# ”A literature review regarding the aetiology, risk factors and treatment involved in oral squamous cell carcinoma”

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# Introduction, prevalence and location in the oral cavity

## Introduction

The aim with this literature review in the field of oral squamous cell carcinoma (OSCC) is to present adequate information about the prevalence, aetiology, risk factors and treatment regarding the disease. Attached to this review is a more specific literature study regarding the role of human papilloma viruses (HPV) in the development of OSCC. Also an attached detailed project plan description regarding the role of HPV and OSCC in a population in Karachi, Pakistan will follow.

This literature review has been performed by collection of review articles in the subject found on PubMed (www.pubmed.gov) and MedLine (medline.cos.com), both which are online research data bases. The key search words we have used are "oral squamous cell carcinoma", "treatment and oral squamous cell carcinoma", "HPV and oral squamous cell carcinoma", "tumour biology", "tumour pathology", "risk factors and oral squamous cell carcinoma" and "oral squamous cell carcinoma and rehabilitation". We have limited our search to only include review articles, articles in English and articles with links to full free text reviews.

Additional to review articles found on the online research data bases we also collected material from various textbooks in the field oral cancer, oral pathology and cellular molecular biology. All references to these books are found in this literature review. Statistical information regarding prevalence and mortality rates in Sweden has mostly been gathered from the Swedish cancer foundation and the oncology centre in Stockholm-Gotland, Sweden.

The information collected is summarized and presented in this literature review as follows; The topics are the prevalence of OSCC, localisation of OSCC in the oral cavity, risk factors that may play a role in the development of OSCC, genetics and oral pathology, diagnostics and treatment, rehabilitation and reconstruction, mortality rates and the dentists role in early detection of OSCC. The more specified review on the role of HPV in the development of OSCC will handle areas such as HPV pathogenesis, prevalence of HPV infection and the use of prophylactic HPV vaccines as protection against cervical cancer and OSCC induced by HPV infection.

**Prevalence**

In the United States approximately 3 % in men and 2 % in women of all the cancers diagnosed are represented by oral malignancies (1, 2). These numbers are present also in Sweden (3).

The prevalence rate is higher among men than women and the median age of diagnosis is over 60 years but the incidence of young adults (age <40) appears to be increasing (2). Studies have shown that the annual incidence of oral cancer in African American in this group (both men and women) is higher than among Caucasians (men and women). The highest incidence rate is among African American males (20.5 cases per 100 000) (4), however over the age of 60 the incidence is higher in white males (40 cases/ 100 000) (37). The diagram in Figure 1 shows age and numbers of cases with OSCC per 100 000 persons in the United States in Caucasians and African Americans.

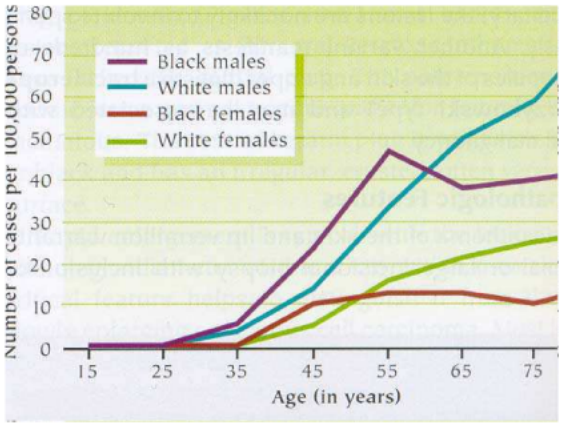



*Figure 1 ( Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company, 2002)*

Risk factors that increase the prevalence rate seem foremost to be the use of tobacco and consumption of alcohol. Risk factors that seem to play a less well understood role are infection with human papilloma viruses (especially HPV-16 and HPV-18), living in rural areas, socio-economic status, poor oral hygiene, ill-fitting dentures, immunosuppressive diseases and various anaemic diseases (1).

Of all the cancers in the oral cavity OSCC is the most frequent one. Approximately 90 % of all cancers in the oral cavity are diagnosed as OSCC (5). The remaining 10 % are for example verrucous carcinomas, spindle cell carcinomas and cancer of the minor salivary glands (2,5).

**Location in the oral cavity**

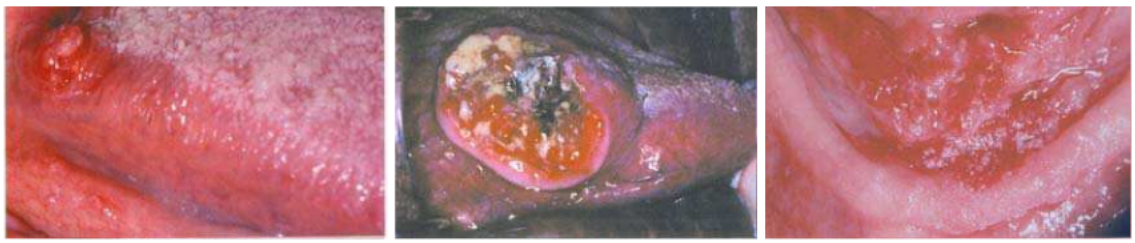
The mucosal surfaces in the oral cavity, as defined by the American Joint Committee on Cancer (AJCC) are divided in to eight areas: the mucosal lip, the buccal mucosa, the lower alveolar ridge, the upper alveolar ridge, the retromolar gingiva, the floor of the mouth, the hard palate and the anterior two thirds of the oral tongue (2). According to the national cancer data base (NCBD) and the U.S. Surveillance, Epidemiology and End result program (SEER) the oral tongue is the most common location of oral cancer (2). Together with the floor of the mouth and the mucosal lip these locations stands for more than 70 % of the diagnosed cases of oral cancer (2).

**OSCC of the oral tongue and the floor of the mouth**

Cancer of the tongue and the floor of the mouth (FOM) represent over 50 % of the oral malignancies, it appears more frequently in men than women between 60 and 80 years of age and especially in heavy alcohol consumers and tobacco smokers (2,1) . The greatest part of the tongue cancers arise from the posterior lateral surface. More than 94 % of oral malignancies of the tongue and the FOM are represented by OSCC (2). Early tongue cancers usually arise within leukoplakias and/or eurytroplakias and are in most cases asymptomatic. Progressive growth eventually results in surface ulceration and muscular invasion (2). In this stage the lesion may cause the patient pain and limitation of tongue motion. Growth can proceed into the floor of the mouth and also posterior into the base of the tongue. Similarly, FOM are often asymptomatic until submucosal invasion or ulceration occurs (2).

As mentioned earlier, on the tongue the lesion most often occur on the posterior lateral border and ventral surface (4). First it can be explained by the fact that carcinogens will mix with the saliva and contaminate these sites before leaving the oral cavity. Second and most likely these regions of the oral cavity are covered by a thinner non-keratinized mucosa which provides

less protection against carcinogens. From these sites the tumour can metastasize and involve the submental nodes, and tumours from the tongue often metastasize to the cervical nodes (4). Picture 1 shows OSCC of the lateral border of the tongue and the floor of the mouth.



*OSCC of the lateral tongue* *OSCC of the latera tongue* *OSCC of the FOM*

*Picture 1 (Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company)*

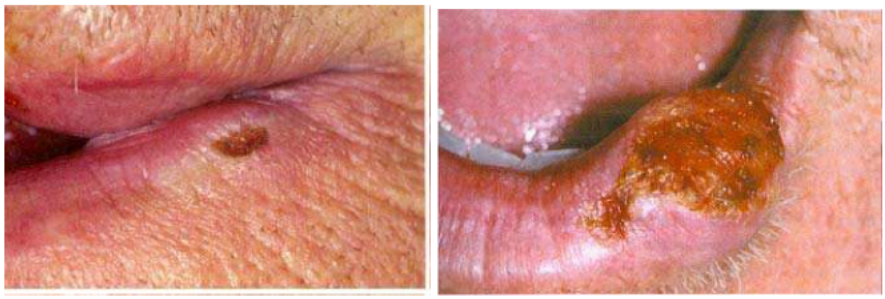
**OSCC of the lip**

Cancer of the lip constitutes 19.8 % of all oral malignancies registered in the National Cancer Data Base (NCDB) (2) and appears most commonly between 50 and 70 years of age and affect men much more than women (37). 87.4 % of the lip cancers are diagnosed as OSSC according to the NCDB (2). OSCC of both the lower and upper lip should be separated from each other. There is a discrepancy between lower and upper lip in development of OSCC. OSCC of the lower lip is far more common than upper lip carcinomas. It is recognized that ultraviolet light and pipe smoking have a much more important role in development of carcinomas of the lower lip than the upper lip, as well as the fact that lower lip carcinoma grows slower than upper lip carcinoma. The prognosis for lower lip carcinoma is better then carcinomas of the upper lip (37).

The etiological agents and risk factors responsible for OSCC of the lips has been the subject to numerous epidemiological studies. They have found that individuals with outdoor occupations and chronic sun exposure have a significantly higher rate of lip cancer than the general population (2).

A Finnish study showed that farmers, foresters and fishermen were five times more likely to develop lip cancer than individuals which performed indoor work (2). Lip cancers are diagnosed earlier than other cancers in the oral cavity because of their location. Lip cancer

typically develops in areas of actinic cheilitis as a slowly growing lesion that enlarges over several months (2). Atrophy of the vermilion border and a pattern less appearance of the lip mucosa may be seen and consisting of fissures, crusts, scales and erythema. Erosion and ulceration occurs in a later stage. A developing cancer appears as an indurated, non-healing ulcer or less commonly as an exophytic mass (2). Picture 2 Shows OSCC of the lower lip.

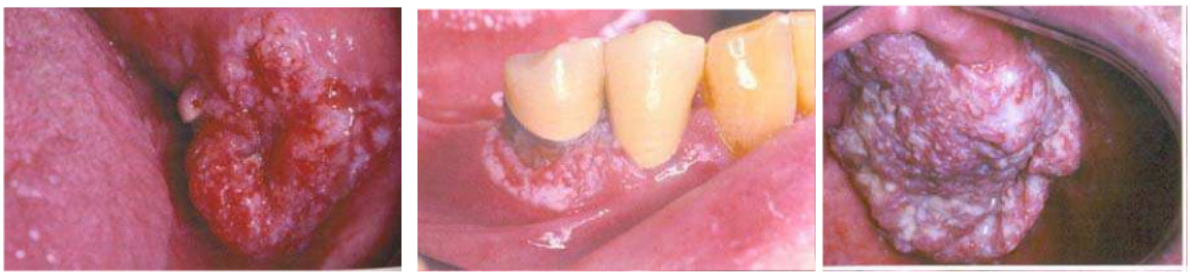


*OSCC of the lower lip*

*Picture 2 (Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company)*

**OSCC of the buccal mucosa and the gingiva**

OSCC of the buccal mucosa and the gingiva represent approximately 10 % of the OSCC in the oral cavity. As in all the other locations men around 70 years of age are over representative and smokeless tobacco is believed to be the main risk factor presenting a clinical appearance that varies from a white patch to a non-healing ulcer to an exophytic lesion (37). Picture 3 shows OSCC of the buccal mucosa and gingiva



*OSCC of the buccal mucosa* *OSCC of the attached gingival* *OSCC of the gingiva/hard palate*

*Picture 3 (Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company)*

# Risk factors regarding the development of oral squamous cell carcinoma

The carcinogenesis behind the development of OSCC is not today fully understood but a number of possible risk factors have been sorted out, and may have a greater or a less effect on the patient itself when taking the individual conditions into consideration. It is clear though that OSCC is a multifactor disease and that different combination of risk factors increases the risk of malignant development significally. The most obvious risk factors known today is the use of tobacco and alcohol combined which seems to have a great increase in the risk of development of oral cancer (6). Other risk factors suggested have been various types of viruses and especially the human papilloma virus and some forms of mucosal diseases like oral lichen planus. The role of these factors is not yet fully understood but seems to play a role in malignant transformation in the oral cavity. In this chapter the most recognized risk factors for oral cancer are discussed with an attempt to estimate their role in development of OSCC alone and in combination with other supposed risk factors known today.

## Smoking tobacco

In regular cigarettes more than 300 carcinogens have been identified where the most studied are the aromatic hydrocarbon benz-pyrene and the tobacco specific nitrosamines. These carcinogens act locally in the oral cavity and the rest of the aero digestive tract where they produce components which interfere with DNA replication leading to damage on the replicating cells which can lead to malignant transformation (6).

Smoking cigarettes has for a long time been associated with a number of cardiovascular- and malignant diseases where OSCC is one of them. The role of smoking tobacco as a risk factor in the development of OSCC is well established through epidemiological studies where it has been shown that the risk for smokers to develop OSCC is five to nine times greater than for non-smokers, and smokers consuming more than 80 cigarettes per day have an increase risk to develop OSCC with as much as 17 times compared to the non smoking control group (4).

Patients suffering from OSCC are significally smoking more than the general population. In a large study conducted in San Francisco, USA one of the results was that roughly 72% of patients with OSCC were smokers, which are two to three times higher than the smoking incidence of the general population thus showing the high risk of smoking cigarettes and the risk of malignant formations (7).

Another well established association is that patients treated for OSCC who continue to smoke cigarettes have a two to six times greater risk of developing a secondary tumour during the first 5 years after treatment compared to patients who stopped smoking after the first treatment (8). Patients who stopped smoking had the same rate of secondary primary OSCC as patients who never smoked (7).

Cigarettes are the most studied form of smoking tobacco and their role in the development of OSCC is unquestionable. Other forms of smoking tobacco such as pipes and cigars have not been studied to the same extent concerning their role of malignant transformation in the oral cavity. However, similar carcinogenic agents are present in pipes and cigars and the studies conducted show an association between the use of cigars and pipes and oral cancer (7).

**Snuff and chewing tobacco**

The role of chewing tobacco and snuff in the development of OSCC has long been debated but still there is no consensus. Smokeless tobacco contains carcinogenic agents but its influence on the carcinogenesis appears not as clear as smoking tobacco. In the United States more than 12 million individuals use some form of smokeless tobacco for example chewing tobacco, and the long term use of these products have been associated with OSCC. A report on 201 oral cancer patients in the United States showed that 23% of the patients used some sort of smokeless tobacco where 50% of those patients reported that they used to place their tobacco at the site of the malignant development (7). American snuff and chewing tobacco have shown some correlation with OSCC but to what extend is not yet clear and further studies are needed in this field.

The Swedish form of snuff has also been debated whether it is a risk factor for malignant development in the oral cavity as it has been claimed for American snuff. However, a recent

case-control study of oral cancer in Sweden has failed to show any association, probably because Swedish snuff is not fermented and contains much lower levels of nitrosamines than the fermented tobacco used in the United States does (9).

Even though there is not any clear association between smokeless tobacco and the development of oral cancer, smokeless tobacco should not be considered an alternative to smoking tobacco. The reason being that its role is not yet fully understood and it does contain carcinogens and affects other parts of the body in a negative way why the recommendation is that both smoking tobacco and smokeless tobacco should be avoided.

**Pan and betel quid**

Chewing pan or betel quid are common in the Indian subcontinent and in some parts of south East Asia. Pan contains betel quid which are prepared from the areca nut and then sun dried and chopped and then placed on a leaf together with slaked lime and usually tobacco. The package is then chewed on in the mouth. The betel quid itself has a slight euphoric effect and is addictive. The slaked lime is used to lower the pH in the oral cavity which accelerates the release of alkaloids both from the betel quid and the tobacco giving an enhanced effect (6).

The use of betel quid and pan is strongly associated with the pre-malignant chronic disease oral sub mucous fibrosis (10) which is discussed later in this chapter.

**Alcohol**

The heavy use of alcohol has long been considered a major risk factor for malignant development in the upper aero digestive tract and in other organs of the body. During the years a number of studies have shown the relationship between OSCC and the heavy use of alcohol consumption, moderate to heavy drinkers have a three to nine times greater risk of developing OSCC than the control group (4). Patients consuming more than 100 grams of pure ethanol per day are classified as extremely heavy drinkers, and have accordingly to a French study increased their risk of malignant transformation in the oral cavity as much as 30 times (11).

It has been discussed if it is the ethanol in the beverage that is carcinogenic itself, but recent studies suggest that the ethanol works more indirect and supports more direct carcinogens from the environment. Especially the indirect support of alcohol on carcinogens from tobacco has been shown to enhance the risk of malignant transformation (6). Nevertheless alcohol consumption alone is after adjustment for other carcinogenic uses like smoking a risk factor of its own (6).

As stated above, alcohol may have a supporting role for other carcinogens and making them more dangerous. Especially the synergistic effect of heavy alcohol consumption and smoking has shown to have a significant increase on the risk of developing oral cancer. It is thought that the alcohol makes the mucosa more accessible for the carcinogens from the tobacco. In the French study mentioned earlier it is demonstrated that patients who are both heavy smokers and drinkers may have over one hundred times greater risk of developing oral cancer than the control group in the study, which implicates the synergistic effect the combined use of alcohol and tobacco may have on the development of OSCC (11).

**Human papilloma virus**

Infection of the oral mucosa with HPV and especially HPV-16 and HPV-18 is thought to be a risk factor in the development of OSCC but not to the same extent that is present in cervical cancer development. A more thoroughly review on the role of HPV and OSCC will follow later in this literature review.

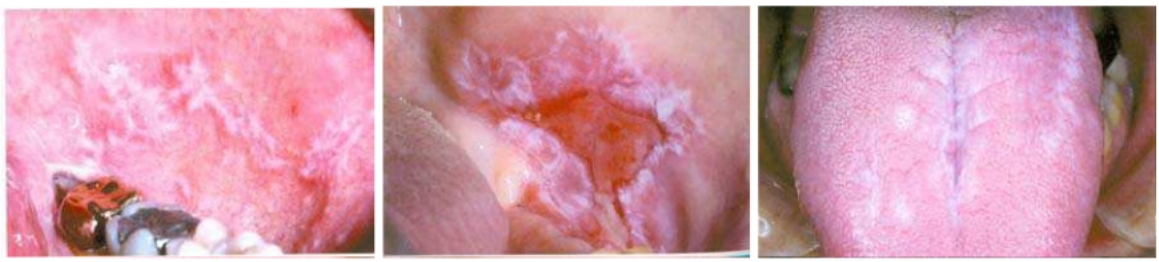
**Oral lichen planus**

Oral lichen planus (OLP) is a relatively common chronic inflammatory disease affecting roughly 0.5% to 2.2% of the general population and usually manifests as a leukoplakia on the buccal mucosa (5). There are several types of OLP where the reticular OLP, erosive OLP and plaque OLP are the most distinctive types (5). The causative factor for developing OLP is still unknown but it is thought to be an immunopathologic disease. OLP usually affects men and women between the age of 30 and 70, while younger and elderly patients are rarely affected

by this condition (5). There is no general treatment for OLP, usually symptomatic treatment and corticosteroids are used to ease symptoms from the disease.

Studies conducted have associated OLP with development of malignancies, especially the erosive LP has been connected with this malignant transformation but it is still debated if OLP should be considered a premalignant lesion or not (5). In two large studies of patients with OLP it was shown that the risk for malignant transformation was 1.2% respectively 1.7% (17,18). The mean time duration after the diagnosis of OLP in these studies to the transformation of carcinoma was close to 6 years. The prevalence of oral cancer in the two studies exceeded the expected ratio in a comparable population.

Even though some studies have shown the association between OSCC and OLP there is no consensus whether OLP should be considered a premalignant lesion, but dentists should carefully evaluate this conditions with regular check-ups so if a malignant transformation occurs it is spotted as early as possible. Picture 4 shows reticular OLP, erosive OLP and plaque OLP



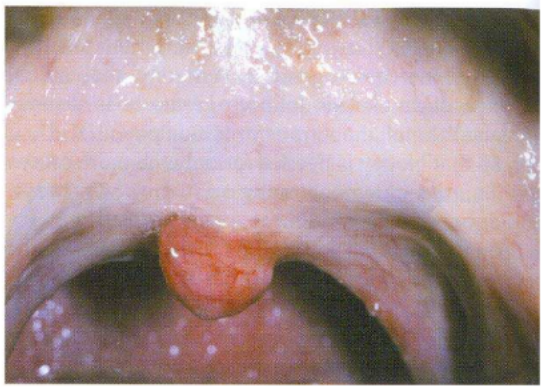
*Reticular OLP* *Erosive OLP* *Plaque OLP*
*Picture 4 (Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company)*

**Oral submucous fibrosis**

Oral submucous fibrosis (OSF) affects various sites in the oral cavity like the buccal mucosa, lips and soft palate. The tissue is symmetrically affected and appears firm, pale and atrophic. The aetiology behind the disease is unknown but it has been linked to the use of betel nut and large consumption of chilli pepper, and therefore the disease is most common in countries like India, Pakistan and Burma where the use of betel nut is more common than in other parts of the world.

Histopathologically the disease is characterized by a chronic inflammation of the submucosal connective tissue, which is followed by a diffuse progressive fibrosis and atrophy of the overlaying epithelium. This atrophic epithelium has a greater tendency to develop epithelial dysplasia which can further develop into OSCC (5).

Clinical symptoms of this disease is a whitening and marble-like mucosa and the progressing fibrosis of the mucosa lead to a reduced mouth opening and difficulties with swallowing and speech. In severe cases OSF is generally considered as a risk factor for the development of OSCC (5). For example in India one study showed a malignant transformation rate of 7.6% of the patients suffering from OSF (4). Picture 5 shows OSF in a betel quid chewer.



*OSF in a betel quid chewer*
*Picture 5 (Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company)*

**Oral hygiene and dental status**

Whether oral hygiene and the use of ill-fitting complete/partial dentures is a risk factor for the development of OSCC has long been debated and the studies performed have been inconsistent in their results.

A newly published multi centre study, which is the largest study to date addressing the issue of oral hygiene and head- and neck cancers, indicates that periodontal disease as defined by poor condition of the mouth and missing teeth may be an independent cause of head-, neck- and oesophagus cancer alone when the factors of tobacco use and alcohol consumption has been adjusted for (19). The association between periodontal disease and head and neck cancer is in the study explained as biological plausible because of the release of proinflammatory

cytokines during the periodontal infection, and the host response to bacterial inflammation are known to play a role in the development of cancer(20).

A Swedish study conducted in 2005 found similar results that average and poor oral hygiene were independent risk factors for the development of OSCC (21), while other studies shown little or no relation between poor oral hygiene and the risk of development of OSCC (22,23,24).

The role of ill-fitting complete or partial dentures as an independent risk factor for OSCC has also been debated mainly because some carcinomas develop in areas covered by dentures. In the Swedish study referred to above (21) they assessed the condition and function of the dentures and found that poorly fitting complete dentures were a significant risk factor for OSCC, explained partly by the damaged caused on the oral mucosa by the denture. However, as stated above it could also reflect ignorance regarding dental care from the patient why these values are hard to interpretive. On the other hand other studies have shown no difference between denture wearers and control group regarding the development of OSCC (7).

Due to the many perimeters involved in the concept oral hygiene and ill-fitting dentures it is hard to get any evidential based results and as long as there is no consensus whether oral hygiene and ill-fitting dentures is a significant risk factor in the development of OSCC, further studies are needed in this complex field.

**AIDS and immunosuppressed patients**

Acquired immunodeficiency syndrome (AIDS) is a predisposing factor for patients to develop both various oral and non-oral malignancies, and one of the malignant diseases that are more common in AIDS patients is OSCC. In patients with AIDS the development of OSCC effects younger patients than usual and other carcinogenic factors are not always present (4). Although a patient with AIDS and immunosuppression has a greater risk to acquire OSCC, other malignant forms in the oral cavity are more common like kaposis sarcoma and lymphoma (5).

### Iron deficiency anaemia

It has also been debated that iron deficiency anaemia in combination with dysphagia and oesophageal webs also known as Plummer-Vinson or Paterson-Kelly syndrome is linked with an elevated risk of development of carcinoma in the oral cavity, oropharynx and the oesophagus (4)

## Tumour biology and cancer development

### Genetics and tumour cell pathology

For the body to work properly the tissues in the body must grow and renew itself during the organism's lifespan. The cells in the body must divide itself when new cells are needed, know when to stop dividing and when to undergo apoptosis by the intercellular signal network in the body that controls the growth and maintaince of cells. Cells also have special characters that tell the cell where it belongs and keeps it from entering other parts of the body where it should not be.

Cancer is foremost a genetical disease and is the result of a pathological change in the DNA sequence carried by a damaged cell, usually as a result from the interaction with various carcinogens. This genetic alteration allows the damaged cell to survive and divide when it normally should have been destroyed by apoptosis. By dividing it creates daughter cells with the same genetic alteration that keeps dividing even though they are not supposed to do so, and may loose their progency for their tissue and spread to other parts of the body normally reserved for other types of cells.

Those type of cancer cells are defined by two heritable properties, that they proliferate and divide themselves not regarding the intercellular signals from the surrounding tissue and that they may invade and colonize other tissues of the body. If the cell keeps proliferating and dividing but remains in a single mass in its normal environment and lacks the ability to invade and colonize surrounding tissues, the tumour formed is said to benign (25). But if these

genetic altered cells have the ability to break loose from the primary formed tumour and enter the bloodstream or lymphatic vessels and in other parts of the body form secondary tumours it is said to be malignant. Different forms of cancer requires different properties but generally it can be said that a cancer cell have four characteristics that distinguish them from normal cell behaviour (25).

1) Cancer cells are much less dependant on cell signals from the surrounding tissues that would regulate their growth pattern, apoptosis and division. This is usually due to the fact that cancer cells have mutations in components of the cell signalling pathways, for example a mutation in the RAS-gene causes an intracellular signal for proliferation even though there is no extra cellular signal present for proliferation, which normally is needed to start the proliferation sequence (25).

2) When a normal cell is damaged the regular pattern is to destroy itself by apoptosis. Cancer cells are much less prone to undergo apoptosis due to mutations for example in the p53 gene which acts as a part of a checkpoint mechanism that causes cells either to cease dividing or to undergo apoptosis if their DNA is damaged. If the p53 gene is damaged the mutated cell will continue proliferation and create daughter cells that also lack the ability to undergo apoptosis, due to the transformation in p53 gene or similar genes (25).

3) Normal human somatic cells will only divide a limited number of times before they permanently stop dividing due to the telomeres on the ends of the chromosomes becomes to short. Cancer cells on the other hand have the ability to reactivate the enzyme telomerase that maintains the telomere length in the chromosomes and allows cancer cells to proliferate indefinitely (25).

4) Cancer cells have the ability to invade and colonize other tissue in the body. This is often caused by the lack of specific cell-adhesion molecules such as cadherins that normally holds the cell in its normal environment. They also have the ability to survive in these foreign tissues where normal cells would die, the mechanism behind this survival and proliferation in foreign tissues is still unknown today (25).

The terms oncogenes and tumour suppressor genes have an important role in the understanding of cancer development in a cell. An oncogene is a gene that through genetic alteration has become hyperactive and the gene product is overproduced, for example the production of various growth factors, receptors or like the above mentioned RAS-gene. The normal form of these genes is called a proto-oncogene but when a genetic alteration occurs in their DNA they can turn into an oncogene and cause cellular disorder (25).

A tumour suppressor gene is also important in the understanding of cancer development. Tumour suppressor genes are genes that codes for DNA repair proteins, for mediators of the DNA damage response like the gene p53 or for regulators of the cell cycle. It codes for the vital parts of the cell that if the cell is damaged it will undergo apoptosis or stop dividing. .If a tumour suppressor gene has been damaged through genetic alteration it may loose some or all of its capability to for example code for proteins that activate apoptosis in the cell. If this occurs, a damaged cell can keep dividing and proliferating without the usually stopping mechanism working properly. Oncogenes and tumour suppressor genes plays a major role in the development and understanding of cancer development in a cell (25).

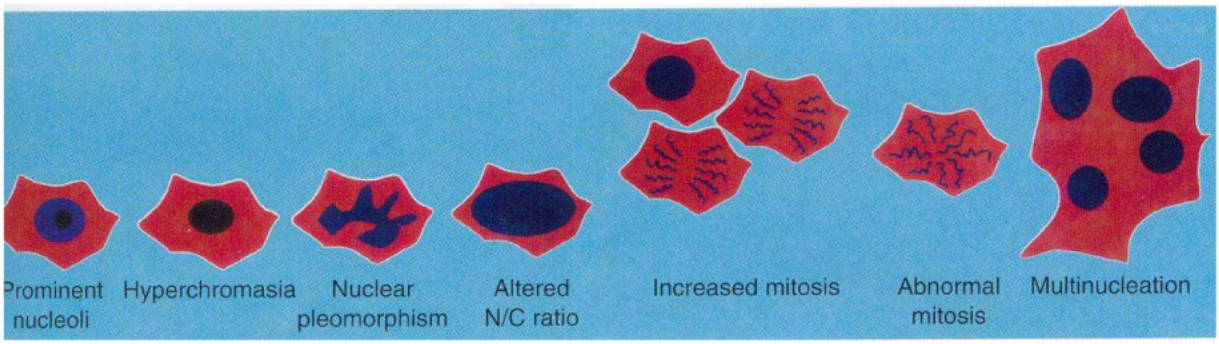
In OSCC and other forms of head- and neck cancers the genetic alterations observed is usually due to oncogene activation and the inactivation of tumour suppressor genes (26). Genetic alterations in the tumour suppressor genes p53 and p16 are frequently observed in OSCC. The p16 gene is like the p53 gene involved in the cell cycle regulation and apoptosis (27).

Studies conducted also show a high activity of the telomerase enzyme in OSCC which keeps the telomeres at the end of the chromosomes in length when in normal functioning cells the telomerase is usually inactive (28,29,30).

These genetic alterations in the DNA sequences create new forms of cells that if they are allowed to keep dividing may cause dysplasia in the tissue.

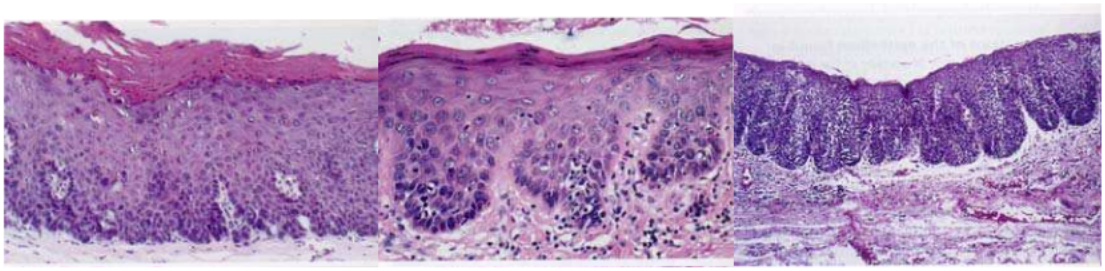
## Epithelial dysplasia

The malignant transformation in the oral stratified squamous epithelium may occur spontaneously without any previous cellular alterations, or develop through gradual alterations in the epithelial cells and its architecture also known as a premalignant stage that may transform into a malignancy through gradual development. Epithelial dysplasia is a premalignant change which is characterized by a combination of cellular and architectural changes and is a histopathological diagnosis (5). Picture 6 shows different degrees in cellular alterations seen in epithelial dysplasia.




*Picture 6 ( Sapp et al. Contemporary oral and maxillofacial pathology 2nd edition. Approved use by Mosby)*

Epithelial dysplasia is based on its histopatological appearance divided into mild, moderate and severe epithelial dysplasia. The division is made after how much of the epithelium that shows alterations, from the surface down to the basal lamina. The stages of an epithelial dysplasia are not fixed and a mild dysplasia can over the years develop into a severe dysplasia or a squamous cell carcinoma, but on the other hand can a mild dysplasia reverse itself if for example the causative agent is removed like the patient stops smoking (5). Picture 7 shows the histological apparences of mild, moderate and severe epithelial dysplasia.



*Mild* *Moderate* *Severe*

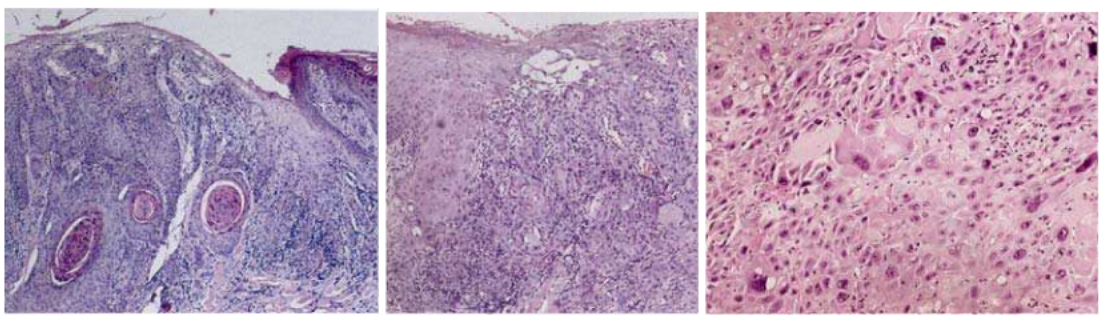
*Picture 7 ( Sapp et al. Contemporary oral and maxillofacial pathology 2nd edition. Approved use by Mosby)*

When alterations in cell structure and architecture are seen in the entire epithelium the dysplasia is staged as a carcinoma in situ (CIS). The only thing that separates this stage from oral squamous cell carcinoma is that the basal lamina still is intact and no spread of dysplastic cells has occurred to the surrounding connective tissue yet, which if it happens would allow for the carcinoma to leave distant metastasis in other parts of the body (5).

**Squamous cell carcinoma**

As mentioned above the histopathological features of squamous cell carcinoma is dysplasia in the entire epithelium and the presence of invasion of dysplastic cells into the underlying connective tissue, which allows the malignant cells to spread to other parts of the body through the lymphatic and blood vessels risking distant metastasis.

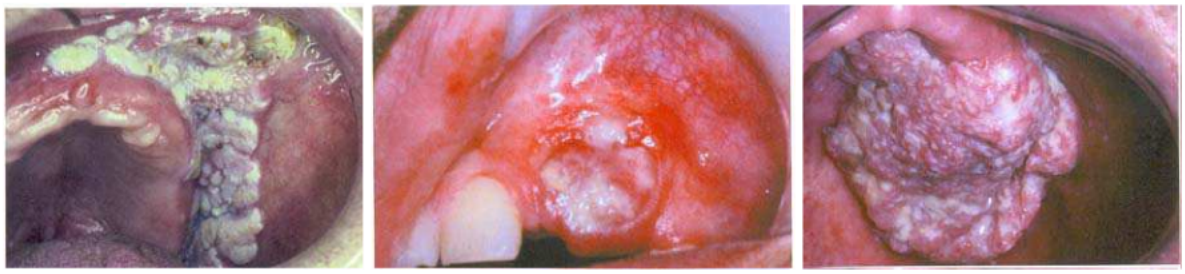
The ability for the tumour to leave distant metastasis is to some extend regulated by the histological degree of differentiation the tumour is showing. A well differentiated carcinoma shows similarities to normal epithelium and has maturation from the basal lamina and produce keratin as normal tissue would. A moderately differentiated carcinoma produces little or no keratin but still to some extend appears as normal tissue. A poorly differentiated carcinoma produces no keratin and has lost the histological pattern seen in normal epithelium. A poorly differentiated carcinoma is more prone to leave metastasis than a well differentiated carcinoma, but other factors like anatomic structures and lymphatic drainage pattern also play a part in the risk of distant metastasis (5). Picture 8 shows well, moderate and poorly differentiated carcinomas.



*Well differentiated* *Moderate differentiated* *Poorly differentiated*

*Picture 8 ( Sapp et al. Contemporary oral and maxillofacial pathology 2nd edition. Approved use by Mosby)*

Clinically epithelial dysplasia, carcinoma in situ and squamous cell carcinoma are usually observed in the oral cavity as a leukoplakia or an erytroplakia which will be discussed in next chapter. Later stages of squamous cell carcinomas can also show features as painless ulcers, indurations or verrucous growth to give a few examples (5). Picture 9 shows an example of clinical appearance by verrucous carcinoma, spindle cell carcinoma and OSCC.



*Verrucous carcinoma* *Spindle cell carcinoma* *OSCC in a severe stage*

*Picture 9 (Neville et al, Oral & Maxillofacial Pathology 2nd edition, Approved use by W:B Saunders Company)*

**Angiogenesis**

Angiogenesis plays an important role in the continued growth of a tumour. Through neovasculation from blood vessels in the surrounding tissue, the tumour supplies itself with the needed nutrients from the blood to be able to continue with its growth (27). It has been shown through clinical trials that a solid tumour cannot grow larger than 2-3 mm in diameter if it lacks the ability to supply itself with blood and nutrients through angiogenesis (3).

The process of angiogenesis is complex and yet not fully understood, but essential for the growth of a tumour. In OSCC, a variety of molecules that is produced by keratinocytes have been suggested to play a part in angiogenesis. Especially interleukin-8 has been suggested to be a major angiogenesis factor (32).

New treatment modalities are under research in order to determine if disrupting the angiogenesis process can be an efficient treatment in cancer patients.

# Diagnostics and treatment

## Diagnostics of OSCC

Diagnosis of a malignant lesion in an early stage is fundamental to minimize the mortality of the OSCC patients. When a suspicious lesion is identified a conventional biopsy using a scalpel or a biopsy forceps are the best way of removing an adequate part of the tissue.

There are other less reliable techniques such as for example an oral brush biopsy© (4). The oral brush biopsy© are delivered in a specific kit and was designed to obtain a complete transepithelial biopsy with minimal discomfort to the patients. Proper use of the brush gives an adequate biopsy sample from all three epithelial layers of the lesion. The sample is scanned by the special computer system that follow with the kit and which are designed to detect oral epithelial precancerous and cancerous cells. Images of abnormal cells are identified and can be shown for a final review by a pathologist (10).

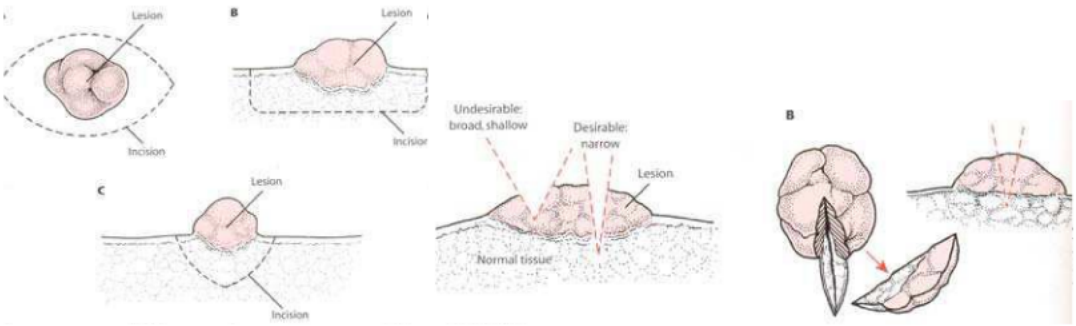
There has been critics who claim that the Oral Brush Biopsy© are given an unacceptably high false positive rate and others who claim the possibility of obtaining a false negative report (10). Further research must be made on the diagnose instrument Oral Brush Biopsy©. However, in the mean time it could be an instrument used for small lesions that are ignored for conventional biopsies (10).

Auto fluorescence techniques have been developed to aid the diagnostics of OSCC. Studies have confirmed that cancer tissue manifest different auto fluorescence spectra compared to normal healthy tissue. The reason of this change is believed to be due to the high concentration of protoporphyrin IX present in malignant tissues (10). This will manifest as a red fluorescence. Further, fluorescence photography is being promoted as an adjunctive diagnostic method for OSCC, although the diagnose should be confirmed with a biopsy (10).

Histopathology of the lesion still remains as the key of which oral cancer is diagnosed. There are many similarities among the lesions that may be present in the oral cavity. Therefore it is important to take all possibilities in to consideration before making a definitive diagnosis.

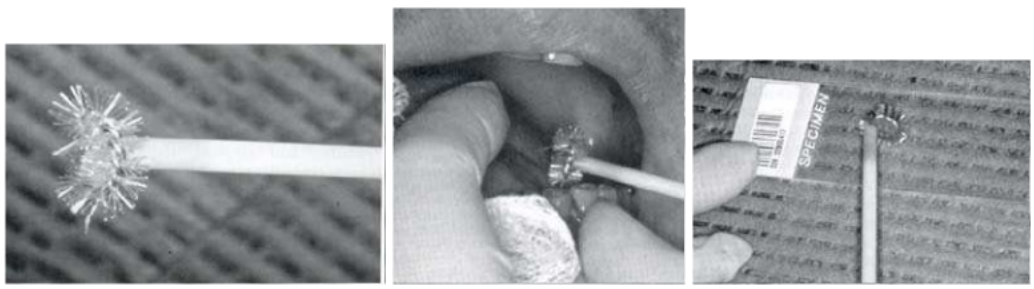
A biopsy can be, as said earlier, made by the dentists and the technique often depends on the size of the lesion. A small lesion can be excisionally removed, while a larger lesion should be incisionally removed with both normal and abnormal tissues (10).

There has been a discussion in what to remove and not to remove when it comes to lesions in the oral cavity. In general, all lekoplakias that shows moderate epithelial dysplasia should be removed compared with lekoplakias which shows an early dysplastic lesion (10). Picture 11 shows exicional biopsy technique and incisional biopsy technique. Picture 12 shows how to handle the oral brush biopsy.




*Exicionaly biopsy technique* *Incisional biopsy technique*

*Picture 11 (Peterson et al. Oral and Maxillofacial Surgery 4th edition, Approved use by Mosby)*



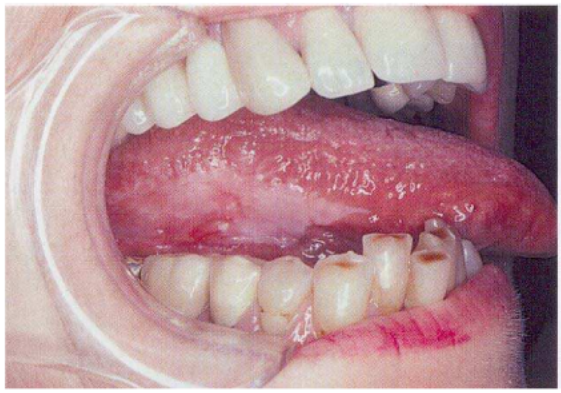

*The use of oral brush biopsy*

*Picture 12 (Peterson et al. Oral and Maxillofacial Surgery 4th edition, Approved use by Mosby)*

**Leukoplakias and Erythroplakias**

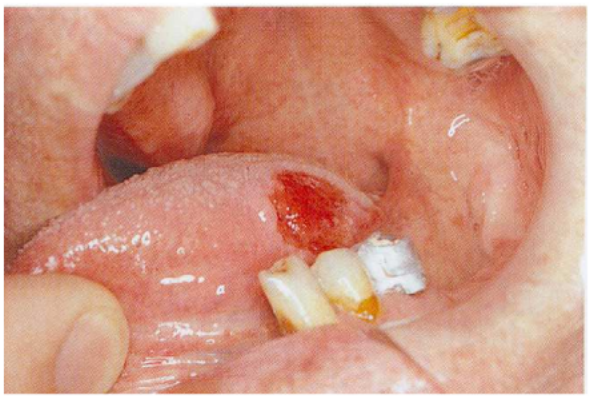
In the oral cavity there might be a clinical appearance such as a white lesion (leukoplakia). These lesions are due to increased thickness of the keratin layer of the epithelium and are characterized by a non removable white lesion. Lukoplakias can be everything from a simple hyperkeratosis to an early stage of an invasive carcinoma (5).

Leukoplakia is a clinical diagnose which can be confirmed if the histopathology shows no other signs. The frequency of dysplastic or malignant changes in oral leukoplakia have ranged widely in different studies, however, in one well known retrospective study which examined 3300 biopsies of white lesions, aproximally 20 % showed some degree of epithelial dysplasia. In this group approximally 3 % were unsuspected OSCC, 4,6 % showed carcinoma in situ and 12,2 % showed mild to moderate epithelial dysplasia (4). Picture 13 shows a leukoplakia with mild dysplasia on the lateral border of the tongue.



*leukoplakia with mild dysplasia on the lateral border of the tongue*
*Picture 13 (Silverman. Oral cancer 5th edition. Approved use by BC Decker Inc 2003)*

Second, there is erythroplakia which also is a clinical term that means “a red patch”. The red clinical appearance is due to a reduction in epithelial thickness and a lack of keratinized layer. As with leukoplakia, erythroplakia is a clinical term which can not be defined pathologically as any other condition (5). Picture 14 shows an erythroplakia that was belivied to be caused by irritation from a temporary crown but turned out as an OSCC.



*Erytroplakia that turned out to be OSCC*

*Picture 14 (Silverman. Oral cancer 5th edition. Approved use by BC Decker Inc 2003)*

Erythoplakia is often shown on the floor of the mouth and on the tongue and are not nearly as common as leukoplakia but shows a higher range of dysplasias or cancers (4). Malignant lesions are often a combination of these two (2).

A more advanced lesion are characterised as a painless ulcer, a tumourus mass or a papillary growth (5). Depending on the stage and local of the lesion it can involve bone, soft tissue, muscles, nerves and even teeth and create symptoms as difficulties of mouth opening, paresthesia and even tooth loss (5).

Leukoplakias and Erythroplakias may be reversible if the irritation which causes the lesion is removed (4). There is a point to wait for the histopathological lab result that confirms a dysplastic change before going further with a final treatment plan. It is always a risk of complications when for example surgery is performed.

**Staging**

Staging of oral cancer is a way of classify the tumour in different aspects. It is determined by physical examination supplemented by CT, MRI, ultrasound (US) or plain radiographs. The staging is used with the system TNM (tumour, nodule, metastasis). Picture 15 shows the TNM staging system.

| Stage | Definition |
|---|---|
| *Primary tumor (T)* | |
| TX | Primary tumor cannot be assessed |
| T0 | No evidence of primary tumor |
| Tis | Carcinoma in situ |
| T1 | Tumor 2 cm or less in greatest dimension |
| T2 | Tumor more than 2 cm but not more than 4 cm in greatest dimension |
| T3 | Tumor more than 4 cm in greatest dimension |
| T4 (lip) | Tumor invades through cortical bone,* inferior alveolar nerve, floor of mouth, or skin of face, i.e., chin or nose |
| T4a | Oral cavity: Tumor invades adjacent structures (e.g., through cortical bone,* into deep [extrinsic] muscle of tongue [genioglossus, hyoglossus, palatoglossus, and styloglossus], maxillary sinus, skin of face) |
| T4b | Tumor invades masticator space, pterygoid plates, or skull base and/or encases internal carotid artery |
| *Regional lymph nodes (N)* | |
| NX | Regional lymph nodes cannot be assessed |
| N0 | No regional lymph node metastasis |
| N1 | Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension |
| N2 | Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension |
| N2a | Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension |
| N2b | Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension |
| N2c | Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension |
| N3 | Metastasis in a lymph node more than 6 cm in greatest dimension |
| *Distant metastasis (M)* | |
| MX | Distant metastasis cannot be assessed |
| M0 | No distant metastasis |
| M1 | Distant metastasis |

*Picture 15 (John.W.Werning. Oral Cancer, Diagnosis, Manegement and Rehabilitation. Approved use by Springer 2007)*

Two centimetres increments in size of the primary tumour are used to differentiate between T1, T2 or T3 lesions of the oral cavity (4). While a T4 lesion is when there is patterns of tumour invasion. A T4a lesion is characterised as a lesion which invade through out cortical bone, into the deep musculature of the tongue, the maxillary sinus or the skin of the face (2).

Secondly there is N, which stands for the extent of lymph node involvement. N1 is a single ipsilateral lymphnode 3 cm or less in its greatest dimension. N2 is an ipsilateral lymphnode that is greater than 3 cm in size or the presence of multiple lymph node metastases. A lymph node greater than 6 cm in size is assigned N3 status. The absence of metastasic disease is classified as M0, whereas the presence of metastatic disease is assigned M1 (2).

The TNM system and staging is intended to provide a mechanism for comparing similar groups of patients who may be candidates for particular options (2).

After the tumour has been classified through the TNM system the tumour is then staged as a class 1,2,3, 4a, 4b or 4c as illustrated in picture 16.

| Stage | Grouping |
|---|---|
| 0 | Tis*N0M0 |
| I | T1N0M0 |
| II | T2N0M0 |
| III | T3N0M0 |
| | T1N1M0 |
| | T2N1M0 |
| | T3N1M0 |
| IVA | T4aN0M0 |
| | T4aN1M0 |
| | T1N2M0 |
| | T2N2M0 |
| | T3N2M0 |
| | T4aN2M0 |
| IVB | Any T N3M0 |
| | T4b Any N M0 |
| IVC | Any T Any N M1 |

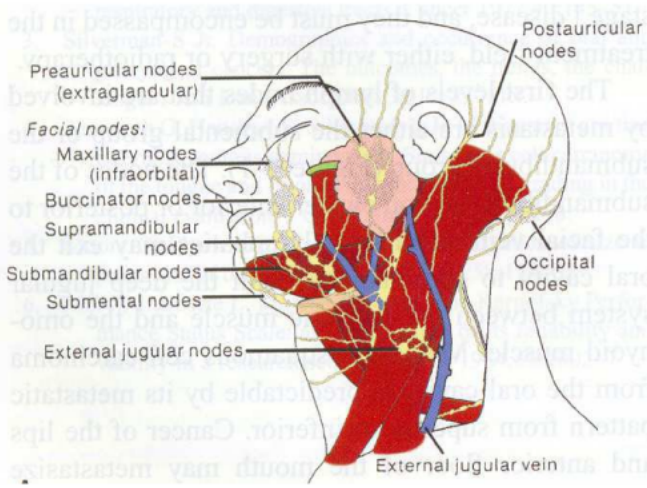
*Picture 16( John.W.Werning. Oral Cancer, Diagnosis, Manegement and Rehabilitation. Approved use by Springer 2007)*

## Treatment of OSCC

The treatment of OSCC is a difficult procedure often leaving the patients with major esthical and functional defects in the oral cavity and face. The most common treatment today for OSCC is radical dissecting surgery and radiotherapy, either alone or in combination (4). The selection of treatment type is determined by the tumours location, size and proximity to the jaws. More advanced stages of tumours usually requires both surgery and radiation, and sometimes including chemotherapy treatment as well (4).

Apart from treatment of the primary tumour the risk of distant metastasis in the lymphatic nodes must be taken into consideration while planning the patient's treatment. Metastasis to the lymphatic nodes occurs commonly but is not always clinically present at the time of treatment. During the treatment planning the risk of lymphatic metastasis is evaluated and if the risk exceeds 20%, treatment of the neck is indicated as well as treatment of the primary tumour (7).

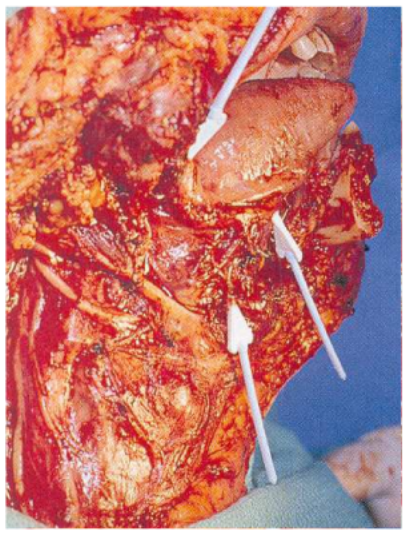
The way OSCC metastasis can be predicted. OSCC in the floor of the mouth usually metastasis to the submandibular region and its lymphatic nodes, while posterior OSCC usually metastasis to the deep jugular lymphatic nodes (7). Anatomical structures are shown in picture 17.




*Anatomical structures of the neck*

*Picture 17 (Silverman. Oral cancer 5th edition. Approved use by BC Decker Inc 2003)*

If the risk of lymphatic metastasis is determined high the neck is treated prohylactically with either radiation therapy or neck dissection. Neck dissection is generally indicated when radiation therapy has failed and when the tumour is classified as a stage 3 or a stage 4 tumour (7). Picture 18 shows the treatment during neck dissection.



*Neck dissection*

*Picture 18 (Silverman. Oral cancer 5th edition*

*Approved use by BC Decker Inc 2003)*

## Surgery

Local transoral surgical removal of the tumour can be conducted if the tumour don't exceed a diameter of 2 cm, otherwise a transcervical approach to the tumour is often needed. The golden standard with surgery is to have a 2 cm healthy tissue around the malignancy when it is removed to minimize the risk of reoccurrence of the tumour (7).

When the tumour is located in the buccal mucosa and due to the appearance of the tumour and how invasive it has been, different surgical treatments are used from minor transoral excisions to radical surgery involving the whole cheek (7).

Superficial OSCC in the buccal mucosa that has not yet spread to the underlying m. Buccinator usually is surgically removed transorally down to m.buccinator and sometimes includes partial removal of m.buccinator (4). More invasive tumours that extend down through the m.buccinator and out in the buccal space usually requires a full cheek resection leaving the patient handicapped both estitically and functionally through the loss of n.facialis (4).

In cases when the tumour is located on the tongue or in the floor of the mouth, usually an extra oral surgical excision is made mainly because the approach through for example an cheek flap gives better access to the malignancy (4).

## Radiation therapy of OSCC

Radiation therapy in the treatment of OSCC can either be used alone or in combination with surgery. Roughly 50 % of all treatment plans for OSCC includes the use of both surgery and radiation therapy (7). When the tumour has grown to the size that the given radiation dose exceeds the tolerance level of the surrounding normal tissue the combination of surgery and radiation therapy is often used. First the main part of the tumour is surgically removed and then the damaged cells and tissue left behind is treated with radiation therapy to minimize the dose needed and protect the normal tissue (7).

The radiation therapy is based on the principle of delivering external beams of x-rays or gamma rays, or by implantation of sources containing beta rays into the tumour site (7). The ionizing radiation kill cells mainly by causing breakage of chromosomes and disrupting the DNA in the nucleus which prevents the cell from dividing further (7). The ionization can cause cell death in both tumour cells and in normal cells but due to biological differences between them the tumour cell is more sensitive to the radiation therapy, thus making it possible to use radiation as a treatment for OSCC without causing to much damage to the surrounding healthy tissue (7).

**Chemotherapy**

The use of chemotherapy in treatment of OSCC can be used either in combination with surgery and radiation therapy, or as an palliative alternative when recidive occurs or when the tumour has left metastasis in the body (7).

A number of chemotherapy drugs have been used in patients with metastasis or recidive of OSCC where the drug methotrexate is the most common in use. Methotrexate has a cytotoxic effect through the inhibition of the enzyme dihydrofolate reducatase who is an enzyme responsible for maintaining the intracellular levels of folates. Folates is needed by the cell for synthesis of purine nucleotides (7).

Chemotherapy is as stated above still more used in the palliative care of a patient than in the actual treatment. The main treatment modalities against OSCC is still surgery and the use of radiation therapy.

New treatment modalities are being tested in the use for treatment of OSCC such as anti-angiogenesis drugs and genetic therapy but to days date the use of surgery and radiation therapy is considered as a first choice in the battle against OSCC.

# Rehabilitation

Patients suffering from OSCC and that have undergone treatment such as surgery, rehabilitation is an important step for the patient to come back to a normal stamina, function and a healthy life.

To increase the survival rate and prognosis and depending on which stage the lesion appears, the surgeon have to be radical in the removing of healthy tissue as well. Therefore it is important that reconstruction surgeons rebuild lost tissue to an acceptable function and an aesthetic look. The oral cavity is still one of the most challenging areas for the reconstruction surgeons. Because of the anatomical structures and the interaction between different part of the oral cavity in function such as speech and swallowing, there is many factors to take into consideration and it is important that every patient will undergo an individual plan before the reconstructive surgeon begin his work of rebuilding the anatomical structures.

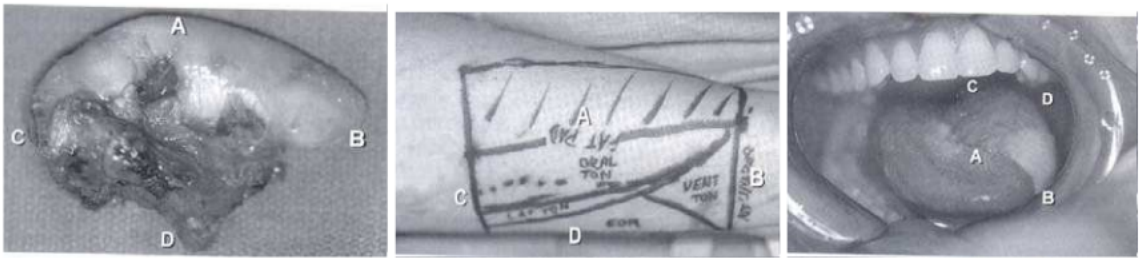
The reconstruction can bee made in different ways and techniques. We are now going to focus on the sites where OSCC appears most frequent. The tongue/floor of the mouth and the lip. And also mention reconstruction of the mandible and the maxilla.

## Reconstruction of the tongue/floor of the mouth

Outcome studies of reconstructive surgery are difficult to make, because of the lack of a standardised system for grading resections and reconstructive modalities. It is difficult to find two defects that are exactly the same and there are also a various numbers of surgical reconstructive techniques which makes it difficult to prove whether a certain reconstructive technique is better suited.

Certain factors are mentioned in the literature. The single most important one is prevention of the function of remaining parts of the tongue. In all studies, independent of which technique used, size of the defect after removal of a tumour affects the ability of how swallowing and speech outcome will be. Defects and tongue resections that are less than one third the size of

the tongue, function is best when repaired with primary closure compared with a pedicle flap or free tissue transfer (3). Picture 19 shows a left hemiglossectomy defect reconstructed with a free tissue transfer using a rectangle tongue template from the radial forearm.




*Free tissue sample from the radial forearm*

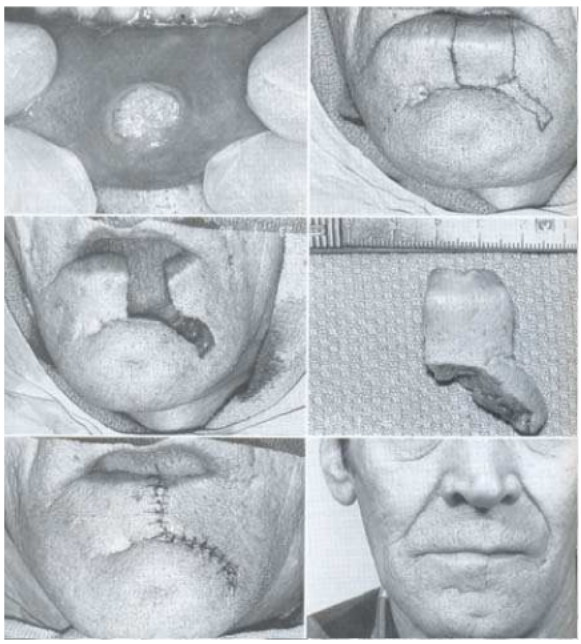
*Picture 19 (( John.W.Werning. Oral Cancer, Diagnosis, Manegement and Rehabilitation. Approved use by Thieme 2007)*

In larger defects the amount of residual functioning tongue is much less and often include surrounding structures which makes primary closure difficult when their is insufficient tissue available to adequately close the larger defects. There is controversy in the literature regarding if free tissue sample is superior to pedicled flaps for large defects considering speech and swallowing. However the results of these two techniques are shown to be much more successful comparing with alternative reconstructive techniques. For example 86 % of patients were able to maintain their nutritional needs and 79 % of spoken words were understandable to untrained listeners with use of the radial forearm free tissue transfer for the reconstruction of tongue and floor of the mouth defects, compared to less than 50 % with alternative reconstruction techniques (3).

**Reconstruction of the lips**

Reconstruction of the lips should follow a number of goals, which are; maintenance of the oral competence, maintenance of an adequate oral aperture to accommodate removable dentures, re-creation of the labial vestibule and preservation of labial sensations. A sensate dynamic flap that restores normal lip height, volume and sphincteric function is preferred and the aesthetic subunits of the lips should be respected (3). As with reconstruction of the tongue

small defects can be reconstructed by local flaps whereas larger defects may require free tissue transfer (3). Picture 20 shows surgically removal of OSCC from the inside of the lower lip and primary closure.



*Surgical removal of OSCC and primary closure*

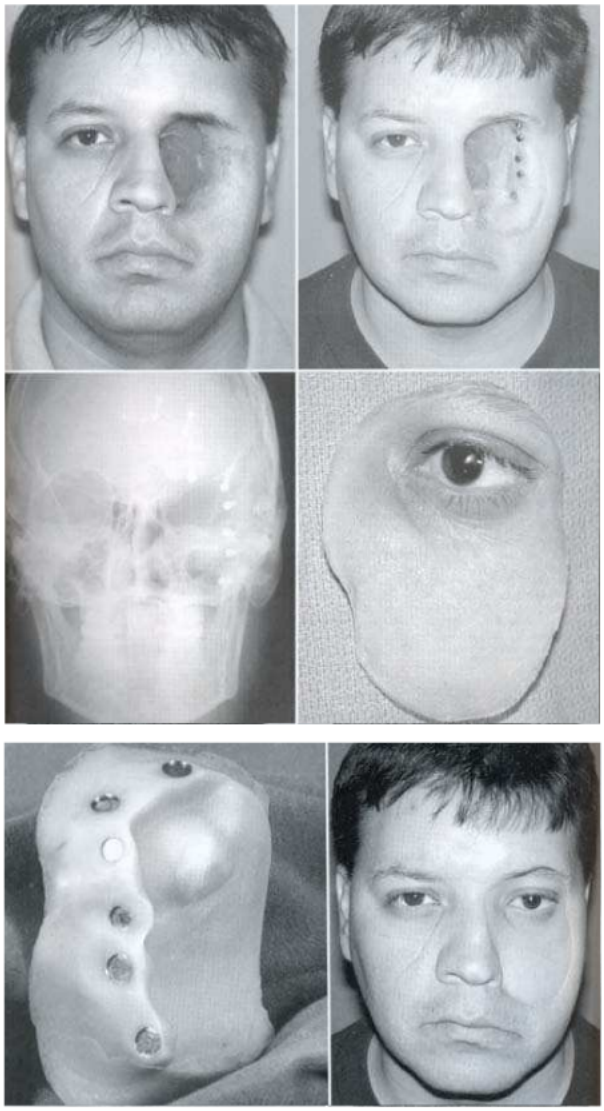
*Picture 20 (Peterson et al. Oral and Maxillofacial Surgery 4th edition, Approved use by Mosby)*

**Reconstruction of the mandible and maxilla**

Refinements in alloplastic materials, microvascular surgical techniques and osseointergration have improved the outcome for most of the patients when a tumour have led to removal of parts of the mandible. Superior outcomes have also been realised by defining the role and limitations of nonvascular bone grafting and free tissue transfer from various donor sites. Historically resection of the oromandibular region often led to defects that had a devastating consequence when it came to the aesthetic appearance and reminded patients about their earlier tumour (3).

The maxilla is one of the regions on the human body which are very complex and have several limitation in terms of advanced plastic surgery. Restoration of the nasal airways, replacement of mucosa with skin and the inability to replace the complex contours of the

midfacial bones and their soft tissues all requires a long and thoughtful multidisciplinary planning to achieve long term functional and aesthetic success (3). Picture 21 shows an Eye and midface implant after surgical removal of pathological tissue.



*Eye and midface implant after surgical removal of pathological tissue*

*Picture 21 (Peterson et al. Oral and Maxillofacial Surgery 4th edition, Approved use by Mosby)*

There are still fields in reconstructive surgery to improve but with new technologies it has given patients a better functional and psychological outcome (3).

Patients who have received different kind of treatment for oral cancer and who develop functional impairment of speech and swallowing have the need of being referred to different rehabilitation specialists. One of them is the speech pathologist. Early referral to the speech pathologist after treatment is critical to achieve a successful functional result (3).

The patients should have the opportunity to meet each member in the interdisciplinary team before treatment is initiated. Different baseline measurement of pre treatment gives information of what the patients can expect of their recovery and to compare changes post treated (3).

## Mortality

As mentioned earlier the chance of surviving OSCC is due to in which stage the tumour is in and what treatment the patient gets access to. For example, 80 % of patients early diagnosed (stage I or II) are likely to survive for 5 years whereas only 20 % who are diagnosed in the more advanced stages will survive for 5 years (36).

The five year survival rate is directly related to stage of diagnosis (4). In the United States 28 900 new cases of Oral Cancer was diagnosed and 7400 died from cancer from the oral cavity 2002. Of these was over 90 % OSCC (4).

In Sweden 221 patients died of oral cancer compared with 709 diagnosed cases 2003. The estimated 5 year survival rate was in this study approximately 60 % and the 10 year survival rate was aproximally 50 % (3).

African American males have higher OSCC mortality than white males in America. This discrepancy between African American males and white males is the fourth highest of all types of cancer in the United States (2). Geographical variations exist all over the world and are most likely correlated to habits related to OSCC (1). World-wide, Oral Cancer accounts for over 127 000 deaths annually (2).

# The Dentists role in discovering of Oral Squamous Cell Carcinoma in an early stage

Most of the patients are seen more commonly by general dentists than by physicians. There for it is important that this category of professional medical health care suppliers gets the adequate education to perform screening examinations of all patients in the diagnosis of oral cancer (4). To be able to deal with patient that can be put in a risk group of developing oral cancer, patients who undergoing oral cancer treatment or rehabilitation of patients, it is important to be aware of the nature of the disease and the impact it has on these patients.

This is to give the best possible care and treatment strategies for the patient (10). It should also be standardised to examine and evaluate the oral soft tissues every time a patient comes for their regular check up. The examination should consist of:

**A medical, family and social history**

Resent and present tobacco use, other habits such as for example alcohol. Resent or present disease involving for example the cardio vascular system, endocrine organs other tumours or cancers in the body or transplanted organs. Any use of medications and drugs such as immunosuppressive drugs.

A study of post transplanted patients in Sweden noted that post transplanted women had a 126 fold increased risk and men a 38 fold increased risk of developing lip carcinoma (10).

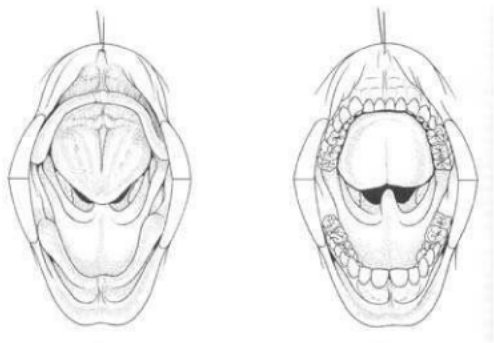
Family history of oral cancer is indeed interesting to know of the patients. One study have for example shown that having a sibling with oral cancer was associated with an increased risk of develop the disease(10). The social history may also provide information in detection of risk patients or as information leading to a diagnose. Again, smoking and alcohol use, frequency and numbers of years of use should be noted (10).

## Visual inspection of the oral cavity and its surrounding tissues

It is important that with the medical, family, and social status in mind make a inspection of the whole patient. Starting with the first appearance, going further with weight, colour, strength and finally look at the oral tissues. Note anatomical architecture, visual examination of the lips, visual examination of the oral mucosa including the floor of the mouth the buccal mucosa, oropharynx, vestibular, the tongue and the gingiva. With this collected information the dentist can find lesions or define how for example tobacco or alcohol habits have affected the oral tissues. By this the dentist can categorise the patients as high or low risk patients for the development of oral malignancies (10).

## Physical examination and palpation

Palpation of tissues are also important to be aware of changes in the tissues providing information concerning changes in texture, physical characteristics of masses, the presence or absence of tenderness and/or indurations and relations between anatomical structures (10). Picture 22 shows areas in the oral cavity that should be visually and physically examined during a check up by the general dentist.



*Picture 22 (Peterson et al. Oral and Maxillofacial Surgery 4th edition, Approved use by Mosby)*

**New technologies for general dentists**

As in all other fields in Dentistry and in the community as a whole new technologies are presented to make our work on a daily basis more effective and in the end of the day give us a better result.

There is no differences in the field in detection of oral malignancies. Different devices have been estimated to help the clinicians with screening of the patients and will now according to the manufactures be an important part in the work on a daily basis at the dental office.

One of these devices is the VELscope ©, commercialised by LED Dental Inc. This device uses a direct fluorescence visualisation (FV). The VELscope produce a cone of blue light which, when directed into the oral cavity excites various molecules within mucosal cells causing them to absorb the light energy and show it as a visible autofluorescence (35).

Healthy oral tissue shows a pale green light, while suspicious tissue, which attenuate the passage of light more appears as dark brown to black (35). The device is not meant to be diagnostic but is a complement to conventional examination. The information can help the clinician to decide whether to take a biopsy or not (35). The British Columbia Oral Cancer Prevention Program following proximally 600 patients with oral cancer or precancerous lesions and have been using the VELscope © as a part of an ongoing longitudinal study. The results using the FV have been very positive. The device detects most of the precancerous and cancerous lesions that was considered to have a high risk of progression and should be treated. The risk of progression of lower grade cancer lesions is less certain but should not be neglected. Of these the device manage to identify a significant portion (35).

Though the device is easy to use the findings of oral malignancies can be challenging. The alternations in fluorescence are not restricted to malignant disease. It can also be a loss of fluorescence in other benign conditions such as lingua geographica, aphthous ulcers and tissue trauma. Therefore clinical training and education programs of using the device is important.

As mentioned earlier new technologies and devices are developed all the time and it is important to have a critical approach to the manufactures commercial, being sure that using a device is evidence based and is supported by scientific studies.

For example the VELoscope © is more likely to distinguish between normal and abnormal tissue where the most commonly of the abnormalities are benign. This makes the result false positive and cause unnecessary stress and fear among patients as well as increasing morbidity through unnecessary surgical biopsy procedures (36).

# Project plan description and evaluation of Human Papilloma Viruses (HPV) role in the development of OSCC

## Introduction

The aim of this literature review is to determine the role of HPV infection in the development of OSCC and to investigate the similarities and differences with the carcinogenesis seen in cervical cancer due to an HPV infection. We also want to discuss current HPV vaccination programs and what protective effect they might have on the oral mucosa. As an attachment to this review on HPV infections a project plan description for the project "A questionnaire and a randomly controlled trial regarding the role of human papilloma viruses as a risk factor of oral squamous cell carcinoma development in a population in Karachi, Pakistan" will be discussed.

The non-enveloped human papillomavirus (HPV) is a circular DNA virus that infects cells in the basal layer of squamous epithelium, where they can either alter epithelial growth and replication or dysregulate the cell cycle, resulting in malignant changes. HPV has been linked to the development of oral and anogenital warts (condyloma) and has shown to be a significant carcinogen in the development of cervical cancer where more than 90% of the cancer cases are related to an HPV infection (12). Today over 81 subtypes of HPV have been identified and sorted after their association with malignancy development as high risk, intermediate risk and low risk HPV (13). Especially the mucosal high risk HPV-16 and HPV-18 involved in the majority of cervical cancer cases have been investigated for decades to learn about their role in the development of OSCC. However, the significant influence of HPV in oral malignancies are still debated (14).

## The pathogenesis of HPV in the oral cavity and the uterus cervix

In cervical cancer a number of high-risk HPVs are known to cause malignant transformation such as HPV-16, HPV-18, HPV-31 and HPV-33. In studies performed regarding HPV infection and OSCC the two only major HPV subtypes believed to play a role in malignant

transformation is HPV-16 and HPV-18 (40). During studies regarding HPVs pathogenesis in the development of cervical cancer it has been found that high risk HPVs expresses oncogenes, for example the oncogenes E6 and E7. These oncogenes disrupts the cell cycle controlling protein p53 thus allowing damaged cells to continuing dividing which can lead to malignant transformation (41). The viral oncogene E6 in high-risk HPVs is the oncogene responsible for binding to the tumour suppressor protein p53 which leads to the rapid turnover of p53 (42). The viral oncogene E7 binds to the retinoblastoma family of the tumour suppressor genes as well as to other proteins involved in the cell cycle (42).

As HPV infected cells divide the viral genome are partitioned into the daughter cells. In normal uninfected epithelia a cell leaves the cellcycle when it leaves the basal layer and this often results in the loss of nuclei in suprabasal cells. Cells infected with the viral genome from high-risk HPV remains active in the cell cycle even after they leave the basal layer due to the action of the oncogene E7 (42).

It is generally believed that HPV infected mucosa in the oral cavity reacts in the same way with oncogene activation as it does in the development of cervical cancer thus leading to disrupted cell cycle control which in the end can lead to malignancies (41).

**Prevalence of HPV infection in OSCC**

A lot of studies have been performed during the years trying to asses the role of HPV and especially HPV-16 and HPV-18 in development of OSCC. Most studies conducted have used different types of molecular analysis to find the presence of HPV infection in oral malignancies with a wide range of results. In an extensive literature review examining the role of HPV in oral lesions the infection was detected in 13.5% of patients with normal mucosa and 26.2% in patients with OSCC (15). In another large study conducted in Sweden it was shown that 36% of the patients in the group with OSCC was positive for a high-risk HPV (81% were HPV-16) while only 0.94% of the control group had an infection with a high-risk HPV (16).

From the wide range of results shown in studies conducted it is clear that OSCC differentiates from cervical cancer where the presence of an HPV infection is necessary for the

development of the disease, and this does not seem to be the case in OSCC. Still the data shows evidence for the presence of a high-risk HPV in some oral pre-malignant and malignant lesions, thus making HPV a possible contributing factor to the development of OSCC. But it is not a necessity in all cases as it is in cervical cancer (14). Further studies are needed to determine the role of high-risk HPV infection and the development of pre-malignant and malignant lesions in the oral cavity using more standardized molecular analysis methods to get consensus regarding the influence of HPVs role in OSCC.

**The role and function of HPV vaccination programs**

Usually cancer formation in the cervix occurs years after the initial HPV infection with high-risk HPV such as HPV-16 and HPV-18 why a therapeutical vaccine against HPV infections would be desirable. At the time of writing no preclinical therapeutical vaccine against HPV has shown any high clinical effect (43). But there has been good clinical trials with prophylactic HPV vaccines and at the time of writing two vaccines are avalible on the market, one from Merck© and one distributed by GlaxoSmithKline©. The prophylactic vaccines distributed by the two companies contains subunit virus-like particles (VLP) composed of a single viral protein, L1, which is the major capsid protein of the virus and contains the immunodominant neutralization epitopes of the virus (43). The vaccines has in preclinical studies shown that when expressed in cells L1 has the intrinsic ability to self-assemble itself into VLPs that can induce high levels of neutralizing antibodies (44,45,46). The vaccine from both companies are given as three intramuscular injections over a 6 month period.

The immunodominant epitopes in L1 VLPs induce neutralizing antibodies that are predominantly type specific for a special subunit of HPVs. Therefore these vaccines are focused on the high-risk HPV that are linked with cervical cancer such as HPV-16 and HPV-18 (43).

The primary results shows a good clinical protection with the vaccines. In a yet unpublished phase three trial from Merck© with an average follow up period of 1.5 years it has been showed that fully vaccinated patients that stays uninfected throughout the vaccination period had almost 100% protection against infection with HPV-16 and HPV-18 (47,48). Other clinical trials have shown a protection against infection with high-risk HPV closer to 90%

(43). More clinical trials and longer follow up periods is needed to fully evaluate the protection given by prophylactic HPV vaccines and to estimate the duration of the protection.

The vaccines used today should give a systemic protection against infection by high-risk HPV and may play a protective role in the development of OSCC. In the time of writing no studies has been found on the effect of HPV prophylactic vaccines and the relation with development of OSCC or tonsillar cancers. It is an interesting field and more studies are needed both regarding the protection against cervical cancer but also to evaluate the effect on protection against malignant development in the oral cavity. Also it will be interesting to follow the trials regarding the development of therapeutical vaccines against HPV which could help offering a large group vital protection against both cervical and other types of HPV induced cancers.

## Project plan description for the project "A questionnaire and a randomly controlled trial regarding the role of human papilloma viruses as a risk factor of oral squamous cell carcinoma development in a population in Karachi, Pakistan"

During the course of our literature review in the field OSCC we got a special interest in HPVs role in the carcinogenesis of OSCC as described above. In our opinion it is a field that needs further research to determine the exact role of HPV in the development of OSCC. During our education we had the opportunity to spend 3 months of education at Altamash Institue of Dental Medicine (AIDM) in Karachi, Pakistan where we got well acquainted with Dr Mohammad Altamash and Dr Dinaz Ghandi who both are oral surgeons. Due to the vast number of OSCC cases in Pakistan we discussed together with them the possibility to conduct a clinical study in Karachi to investigate the role of HPV in the development of OSCC. The aim with this clinical trial was to compare two matched groups, one test group suffering from OSCC treated or untreated and one healthy control group to determine the difference in the presence of HPV infection in the oral mucosa.

The patients planned to participate in this clinical study was intended to be collected from AIDMs regular open practise for the control group and from the oral cancer department of Aga Khan hospital in Karachi for the test group. The two groups would contain roughly 40 patients each and would be matched against each other in gender, age, socio-economic status and oral habits such as the use of betel quid. Each patient would be informed about the purpose of the project both orally and in writing and would have to give a written consent to participate in the study. The patient information sheet is seen on *attachment 1*. This study was given an ethical approval from the ethical committee in Karachi, Pakistan.

After the patients had given the approval on participating in the study they would been given a random number for the protection of their personal data. The patients would thereafter been asked to fill out a questionnaire regarding if they have had the diagnosis OSCC, other medical issues, habits such as the use of tobacco and betel quid and so on. This questionnaire would later be used to rule out biases and for example see correlations with tobacco use. The questionnaire is seen on *attachment 2*.

After the fulfilment of the questionnaire the patients would be asked to leave a non invasive tissue sample from the oral mucosa using an oral brush to remove epithelial cells from the mucosa. The tissue sample would using PCR-technique be investigated for the presence of HPV infection with a special interest in the high-risk HPV-16 and HPV-18.

The results from the test group and control group would be investigated to determine if there is any significant difference in the presence of HPV-16 and HPV-18 between the two groups which could indicate that HPV infection plays a role in the development of OSCC.

Unfortunately the project cost exceeded 7000 USD mainly due to the high purchase costs for reagenses used in the PCR part of the project. Due to the lack of sufficient fundings the project could not be conducted during our undergraduate period. Our hope is that in the future a similar project can be conducted as a part of the student exchange program between Karolinska Institutet and AIDM in Karachi, Pakistan. It would serve both as an important project in the investigation of HPVs role in the development of OSCC but would also strengthen the bonds between Karolinska Institutet and AIDM through research.

# ATTACHMENT 1

## Patient Information

You have been selected to participate in a clinical study and a questionnaire regarding the role of human papilloma viruses in the development of oral squamous cell carcinoma (Oral cancer). This project is in collaboration between Karolinska Institutet in Stockholm, Sweden and Altamash Institute of Dental Medicine in Karachi, Pakistan. The project and its purpose have been ethically approved by the ethical committee in Karachi, Pakistan.

The aim of this study is to through a questionnaire and a brush/saliva sample evaluate the presence of human papilloma virus in a patient group with oral squamous cell carcinoma (OSCC) and a control group not diagnosed with the disease. You will be asked to fill in a questionnaire regarding some aetiology factors known to play a role in the development of OSCC. Some questions are of personal and sensitive nature but the information is strictly confidential and can not be traced to any individual person. Your identity will not be known to the supervisors. All data received from this questionnaire will be used in research purposes only. Secondly, we will kindly ask you to leave a non invasive tissue sample that will be used to detect the presence of human papilloma virus in both the group diagnosed with OSCC and the control group. The sample will be taken through brushing of the oral mucosa and with a rinse collect the saliva containing the tissue sample. This is an easy and non painful procedure and no invasive treatment will be done.

All participating patients that truly fill out the questionnaire and approve of giving a saliva sample will be rewarded 400Rs. Please read this information above carefully and if any questions do not hesitate to ask the supervisors and personnel at hand.
I hereby approve of the information above and accept to participate in this questionnaire and to give a saliva sample for research purposes only.

Name Date

**ATTACHMENT 2**

# Questionnaire

**Patient number (Will be filled in by supervisor):**

_____

## General situation

| | |
|---|---|
| 1) Gender | **Male/Female** |
| 2) Age | _____ |
| 3) Living conditions | **Single/Married/Divorced** |
| 4) Financial situation (subjective) | **Low/Average/High** |

## General condition

5) Do you have any diagnosed systemic disease? **Yes/No**

5) If yes which disease/diseases? (If no leave blank)

_____
_____
_____
_____
_____

7) Do you use any medications? **Yes/No**

8) If yes which medications and for what indication? (If no leave blank)

_____
_____
_____
_____
_____

9) Do you have the diagnosis HIV/AIDS? **Yes/No**

10) Do you have/have you had any form of cancer? **Yes/No**

11) If yes please type which form of cancer (If no leave blank)

_____
_____
_____
_____
_____

12) Do your father, mother, siblings or child ever had/has the diagnosis oral cancer? **Yes/No**

13) Do you have/have you had the diagnosis oral cancer? **Yes/No**

If no skip question 14-15. Go to question 16

14) Which location has/had the leasion in the oral cavity?

**Floor of the mouth/ Gingiva/ Tongue/ The palate**

15) If given treatment, have you had any relapses of oral cancer? **Yes/No**

16) Do you wear complete dentures daily? **Yes/No**

17) Do you wear partial dentures daily? **Yes/No**

| |
|---|
| 18) If yes and diagnosed with oral cancer, is/was the denture in contact with the site of where the lesion developed?(If no use of dentures leave blank)<br><br>**Yes/No** |

## Habits

| | |
|---|---|
| 19) Do you use smoking tobacco? | **Yes/No** |
| 20) If yes, for how long? | **Less than 1 year/1-5 years/5-10 years/over 10 years** |
| 21) If yes, how much? | **Less than 10 cigarettes per day / 10-20 cigarettes per day/ More than 20 cigarettes per day** |
| 22) Do you use Naswar? | **Yes/No** |
| 23) If yes, for how long? | **Less than 1year/1-5 years/5-10 years/over 10 years** |
| 24) If yes, how often? | **Daily/couple of times a week/ Less than once a week** |
| 25) Do you use pan/betel quid? | **Yes/No** |

| | |
|---|---|
| 26) If yes, for how long? | **Less than 1year/1-5 years/5-10 years/over 10 years** |

| | |
|---|---|
| 27) If yes, how often? | **Daily/couple of times a week/less than once a week** |

| | |
|---|---|
| 28) Do you consume alcohol? | **Yes/No** |

| | |
|---|---|
| 29) If yes, how much? | **Once a month/ Once a week/ Every day** |

| | |
|---|---|
| 30) If yes, for how long? | **1-5 years/ 6-10 years/ more than 10 years** |

Thank you for your participation. All information will be handled confidentially and can not be traced to an individual. The information given in this form and the mucosal and saliva sample given will be used in research purposes only. A reward of 400Rs will be given to all patients fulfilling this form and that accepts to give a mucosal and saliva sample for detection the presence of human papilloma viruses.

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