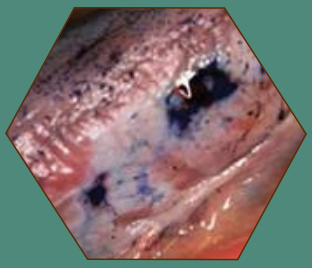
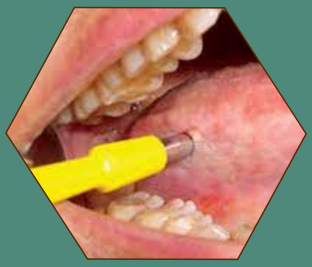
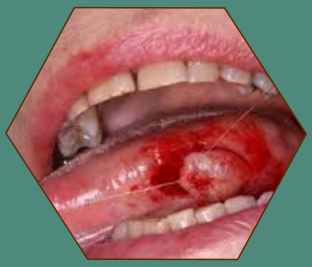
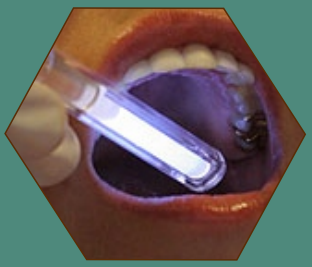
# Handbook of Diagnosis *for* Oral Cancer-Basics to Advanced











**Editor**
**Dr. Mahesh KP**

INNOVATIVE PUBLICATION

# Handbook of Diagnosis for Oral Cancer-Basics to Advanced

IP Innovative Publication Pvt. Ltd.

# Handbook of Diagnosis for Oral Cancer-Basics to Advanced

## Dedicated

To all the parents
To all the teachers
To my dear students

## Contributors

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**Dr. Karthikeya Patil**
**Dr. Aparna V**
**Dr. Naveen Kumar RK**

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**Handbook of Diagnosis for Oral Cancer-Basics to Advanced**

ISBN : 978-93-91208-71-4

Edition : First, 2022

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**Dr. Surinder Singh**
Vice Chancellor

February 25, 2022

## FOREWORD

It gives me immense pleasure in writing the foreword for the '***Hand Book of Diagnosis for Oral Cancer-Basics to Advanced***', authored jointly by Dr. Karthikeya Patil, Professor & HoD, Department of Oral Medicine and Dr Mahesh K P, Reader, of JSS Dental College & Hospital, Mysuru and published by IP Publishers, New Delhi.

Oral cancer is a global challenge with high burden on the society and family. Increasing incidence of oral cancer is to be reduced by awareness to the general public. There is need for the health workers around the world also to be updated on recent advances in oral cancer. Keeping the above reasons in mind, the book comprising of various issues pertaining to oral cancer is written.

When it comes to oral cancer management, two important issues have to be considered diagnosis and management. Diagnosis of oral cancer has come a big way from basics to advanced non invasive and invasive methods. Early diagnosis of oral cancer plays a vital role in the management of oral cancer. It is the accurate or precise diagnosis which will help the clinician to make appropriate decisions on the management protocol.

First four chapters helps the clinician to understand etiology or risk factors, various ways to classify oral cancer and its clinical presentation. Staging of oral cancer is a crucial step in diagnosis as it reports to the oncologist. On the basis of staging, surgeon will decide the treatment protocol. Decision is made on the customized treatment protocol for each of the patients.

Final chapters comprise of numerous methods of diagnosis. It deals with several methods from basic to advance. It may be chosen based on the circumstances and availability of materials.

Hence this book will be useful to clinicians and researchers in the field of oral medicine. We all look forward to future developments in diagnosis paradigms that will lead to even better outcomes for patients with oral cancers, and this textbook will serve as an important point of reference for these future advancements.

I offer my best wishes to the authors for their endeavor and express my heartiest congratulations to them for taking this initiative and publishing this book on MCQs exclusively of Human Anatomy and Physiology. Lastly, I encourage the readers and learners to take benefit of this book and incorporate their learnings in the future.

With best wishes

**Dr Surinder Singh**
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# Preface

*"Knowledge is power, knowledge shared is power multiplied"*

**Robert Noyce**

Oral cancer is a growing and still challenging problem, which is having a high burden on the nation. Early diagnosis and management is the need of the hour. In these aspects diagnosis of the lesion at the early stage will lead to good prognostic results.

The book helps the students and researchers to understand the diversity and their aftermath of diagnosis of oral cancer.

Diagnosis of oral cancer has came a long way from chair side procedures to invasive surgical procedures. The book encompasses the above features in detail, so that the reader is not left with any topic untouched. This book turns all your information to knowledge for making diagnosis to different level in the management of oral cancer.

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# Chapter 1

# Introduction

*Dr. Mahesh K P, Dr. Karthikeya Patil*

Cancer of the head and neck (H and N cancer), including all oral, laryngeal and pharyngeal sites, is the sixth most common cancer. Oral squamous cell carcinoma (OSCC) accounts for about 40% of head and neck and 90–95% of oral malignancies[1,2]. Five-year survival is about 76–80% if diagnosis is performed in stage 1 and 2. Late diagnosis in stage 3 and 4 can decrease this value to 41% and 9% respectively. At this time, metastasis to regional lymph nodes has occurred and cancer cannot be treated without undesirable complications and morbidity. Unfortunately, majority of cases are diagnosed at stage 3 and 4 with more than 50% of them exhibiting metastatic lymphadenopathy. Despite advances in diagnostic procedures in medical practice, mortality of OSCC has remained unchanged in the past 40 years and a significant diagnostic delay (up to 8 months) has persisted over time[2].

There are some reasons for this delay:

1. Lack of general understanding of the signs, symptoms, and risk factors associated with oral cancer.
2. At least half of the population is not subjected to routine oral examinations.
3. Early-stage oral cancer patients show no symptoms.
4. Malignancy in areas of oral cavity that would ordinarily be overlooked by patients and/or doctors.
5. Lesions that are similar to benign lesions.
6. Failure of health care professionals to prevent and diagnose the disease early[2].

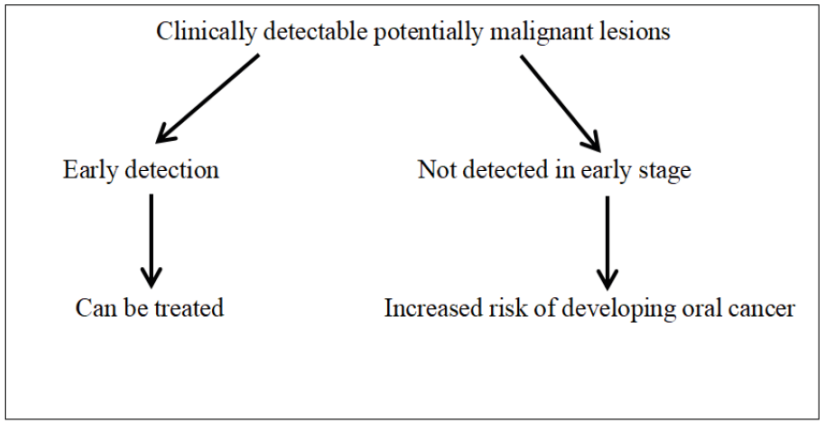
Oral cancer is the most common cancer in India, and it has one of the highest fatality rates of any cancer. The late diagnosis of oral cancer results in a high rate of mortality and morbidity. The therapy and survival rate are influenced by the timing

of diagnosis. Early detection and prediction of the progression of these lesions is critical for providing care to patients and improving their quality of life and survival rate[3]. Hence, here is an overview of the advancements in the diagnosis of oral potentially malignant disorders.

Oral cancer incidence has increased dramatically during the last decade. It is frequently identified in the late stages or when it becomes symptomatic, which has an impact on the individual's survival rate[3]. Because of risk factors such as tobacco and alcohol usage, males are affected at twice the incidence of females. Furthermore, malignant transformation takes years, during which time the lesion can be prevented from progressing. Reducing risk factors such as cigarette and alcohol use can result in the premalignant lesion regressing. The gold standard for diagnosing oral cancer will always be biopsy of the lesion with histopathological examination, which has several limitations[3]. It is time-consuming, expensive, and invasive, and it necessitates the services of a qualified health care professional. The following recent non-invasive developments in detection technologies that can be employed for the early diagnosis of oral potentially malignant disorders to make oral cancer curable and increase patient survival include auto fluorescence spectroscopy, photodynamic therapy, and Raman spectroscopy[3].

The tongue, floor of the mouth, buccal mucosa, gingiva, and lips are the most prevalent sites for oral cancer. Chewing betel, pan, and Areca is known to be a risk factor for oral cancer in several Asian nations, particularly in India. Several studies have been conducted in the past to determine the factors that contribute to the delay in diagnosing Oral Squamous Cell Carcinoma (OSCC), but the rate of early detection has remained frustratingly stable in recent decades. The lack of public knowledge of the many indications, symptoms, and risk factors for oral cancer is thought to be the cause of the long-term diagnostic delay[4].

Improved early diagnosis of oral cancer, either by case detection or organised screening programmes, is one solution to this problem. Oral cancer may be preceded by a clinically evident potentially malignant lesion or begin as a small, circumscribed, typically asymptomatic lesion early in its natural course. These lesions can be treated if detected early enough, avoiding the cancer's very high death and morbidity rates. Increased public awareness regarding the significance of frequent oral screening or case detection to detect small, otherwise asymptomatic malignancies and precancers might help achieve this aim (secondary prevention).[2,5].




**Flowchart 1: Outcome of early detection of potentially malignant lesions.**

Squamous cell carcinoma (SCC), which accounts for 96% of all oral malignancies, is generally preceded by dysplasia, which manifests as white epithelial lesions on the oral mucosal (leukoplakia)[6]. Almost all oral squamous cell carcinomas (OSCCs) are preceded by visible alterations in the oral mucosa, most commonly in the form of white (leukoplakia) and red patches (erythroplakia). Other inflammatory oral mucosa disorders, such as lichen planus, submucous fibrosis, and maybe oral fibrosis owing to systemic sclerosis, have also been linked to an elevated risk of OSCC development. It is thought that identifying and monitoring these malignant lesions and conditions allows clinicians to detect and treat early intraepithelial stages of oral carcinogenesis, such as mild, moderate, or severe dysplasia and carcinoma in situ, which all occur before invasive OSCC develops. Due to the high survival rates of early OSCC, the prognostic implications of detection and treatment of these early intra-epithelial stages of oral carcinogenesis are quite important[1,6].

Leukoplakia was found to develop in 1–4% of the total population. Over the course of five years, malignant transformation occurs in 1–40 % of leukoplakias[6]. Dysplastic lesions that appear as erythroplakia (red lesions) have a 90% chance of turning cancerous. Tumour diagnosis is further confounded by a proclivity for field cancerization, which results in multicentric lesions that aren't always clinically evident[6].

Epithelial dysplasia is widely considered to be one of the most important indicators of malignant progression in premalignant lesions. Premalignant lesions

that are discovered and treated early on are less likely to proceed to cancer. Oral cancer screening can be improved with any method that increases early identification of lesions[7].

## Oral Premalignant Disorders

Oral SCC is caused by a variety of molecular and biochemical cellular changes, as well as changes in the underlying fibrovascular stroma, such as neovascularization. Clinical abnormalities in the afflicted epithelial tissues, known as precancerous lesions, occur in tandem with cellular modifications. Oral precancerous lesions have clinical relevance because of their link to malignant development into OSCC. Precancerous lesions in the mouth can only be detected histologically, and they appear as a spectrum of epithelial alterations rather than separate categories[8].

Precancerous alterations were classified as mild, moderate, severe, or carcinoma in situ by the World Health Organization in 2005. The probability of malignant transformation has been estimated to be between 6.6–36.4%, while a recent meta-analysis found a rate of 12.1%[8]. Clinically, the most frequent precancerous lesions appear as white, red, or a combination of white and red mucosal alterations. These clinical conditions are known as leukoplakia or erythroplakia. Oral lichen planus and oral submucous fibrosis are two other disorders that are considered precancerous. In addition, discoid lupus erythematosus and other uncommon genetic disorders like dyskeratosis congenita and epidermolysis bullosa are less prevalent lesions[8].

The malignant potential of the above mentioned oral lesions cannot be accurately predicted solely on the basis of their clinical characteristics, histological evaluation is essential for all suspicious lesions[8]. But histological findings only indicate that a given lesion may have malignant potential (dysplasia), and cannot be used for the prediction of malignant changes. Thus, the presence of dysplasia only indicates that an oral lesion may have an increased risk of malignant transformation. Molecular biomarkers capable of identifying the subset of lesions likely to progress to cancer are being widely investigated including genetic and epigenetic alterations observed in oral mucosal precancerous lesions[8].

Microscopically, the lesions may exhibit hyperplasia, hyperkeratosis, and acanthosis. A difference between dysplastic and non-dysplastic epithelium must be made histologically. Lesions with degrees of atypia are referred to as epithelial dysplasia precursor lesions. When changes occur in the basal or parabasal

keratinocytes, it is referred to as mild dysplasia; when changes extend to the middle layer, it is referred to as moderate dysplasia; and when changes extend to the surface layer, it is referred to as advanced dysplasia or carcinoma in situ[9]. Besides white patches, there may be red lesions. Erythroleukoplakia is a term used to describe lesions that have a mixture of red and white changes. Dysplasia is quite prevalent in erythroplakias. Because of the high risk of malignant transformation, it should be treated. If a lesion in the oral mucosa does not heal after three weeks, a malignant tumour or other severe disease should be considered, requiring a biopsy and histological investigation[9].

❑

# Chapter 2

# Etiology of Oral Cancer

*Dr. Mahesh K P, Dr. Aparna V, Dr. Naveen Kumar R K*

***Keywords: Tobacco, Alcohol, Bacteria, Virus, Genetics, Radiation***

## Tobacco

In 2007 the IARC concluded that "there is quite evidence to establish that snuff smoke is carcinogenic, and it causes cancer of the oral cavity and pancreas". The risk for developing oral cancer is 3 times higher in smokers compared with non-smokers[9]. Tobacco has a linear dose–response carcinogenic impact in which exposure time is more relevant than exposure intensity[10].

Tobacco usage in whatever form (chewing, smoking, sniffing) damages the oral cavity and can lead to life-threatening disorders including oral cancer. The most crucial step in preventing tobacco-related oral health problems is to stop using tobacco products. When a smoker quits smoking, his or her risk of oral cancer drops dramatically. After ten years of smoking cessation, the oral cavity health of ex-smokers and non-smokers is similar[11].

The chance of acquiring oral leukoplakia and oral cancer is reduced in this instance. Its abolition will have immediate effects, including as longer life expectancy and the avoidance of tobacco-related ailments. Tobacco's harmful consequences are increasing each year, yet, as strange as it may sound, its usage is also increasing. Every year, the average age of smokers decreases, but the prevalence of tobacco use rises in the 13–15 age range[11].


**Fig. 1: Types of tobacco (image courtesy to https://www.piedmont.org/living-better/is-smokeless-tobacco-safer-than-cigarettes, https://www.discovermagazine.com / health/ thirdhand-smoke-study-finds-smokers-clothes-are-carrying-cigarette-chemicals).**

A cigarette-smoking environment is equally dangerous as the risk of oral cancer is 87% higher in people who never smoked but were exposed to a cigarette-smoking environment (involuntary smoking)[10]. This smoke contains various cancer-promoting components that may be divided into three categories: nitrosamines, benzopyrenes, and aromatic amines. Pre-carcinogens are substances that must be altered by oxidative enzymes in a coordinated manner. Aside from oxidation, enzymatic or non-enzymatic metabolism can also create carcinogens, such as free radicals, which have unpaired electrons and are highly reactive, capable of causing mutations through complicated pathways. Snuff consumption exposes the oral epithelium to oxygen and nitrogen free radicals, which can interfere with antioxidant defence systems. These free radicals are discovered in high concentrations in oral precancer and cancer. The frequent development of oral carcinomas along the lateral and ventral sides of the tongue, as well as on the floor of the mouth, may be due to the pooling of saliva carrying carcinogens in low parts of the mouth. Smokeless tobacco use has also been associated with Oral Cavity Carcinoma[9,10].

A significant risk of oral cavity-related death has been documented as a result of cigarette usage. As a result, smoke is recognized as a significant risk factor for various illnesses as well as probable precipitating factors, such as oral leukoplakia. Leukoplakia is a condition that frequently precedes oral cancer and has a similar

origin. That is a response to chronic irritant factor like tobacco. For most people, removing the source of irritation, such as stopping tobacco or alcohol use improves the pathologic condition[11].

## Arecanut

Arecanut is the seed of the Areca catechu fruit of the oriental palm. It's the main component in a variety of widely used chewed products. Natural or processed nut slices can be blended with a number of ingredients, including slaked lime and spices like cardamom, coconut, and saffron. They can be blended with tobacco products or wrapped in betel leaves[12].

It has been shown that "areca nut only users" have a higher chance of developing oral cancer. Tobacco products mixed together with other ingredients raises the risk of oral cancer. Areca consumption increases the risk of cancer for both the duration and daily frequency of use, implying a dose-response relationship. Areca products' role in causing oral fibrosis and cancer, as well as likely contributing to other diseases, has aroused serious public health concerns[12].

## Alcohol

For nearly half a century, alcohol has been recognized as a significant risk factor for oral cancer, particularly when combined with cigarettes. Approximately 75% of all oral malignancies are linked to the use of alcohol and cigarettes[13].

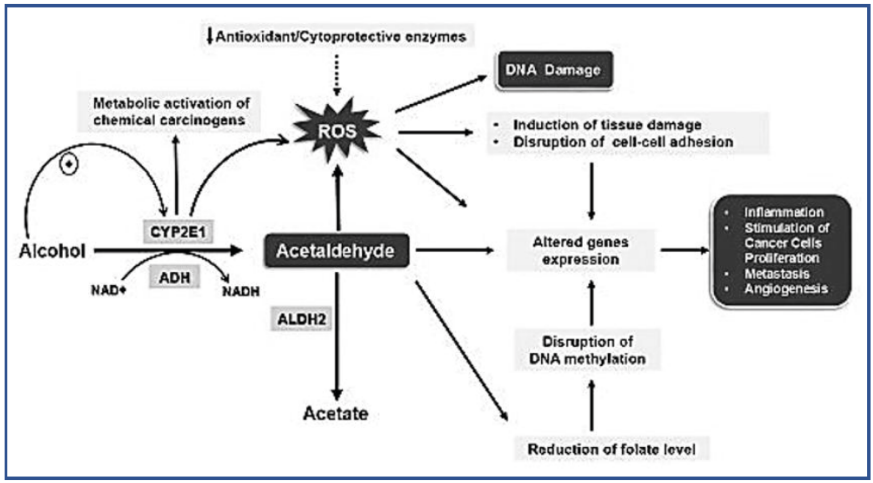
Alcohol which is another etiologic agent is an important promoter of carcinogenesis. The most important clinical effect of alcohol appears to be that it potentiates the carcinogenic effect of tobacco at all levels of tobacco use, albeit this effect is most noticeable at the greatest levels of alcohol use[10].


**Fig. 2: Alcohol is another risk factor for oral cancer (image courtesy to https://www.cookinglight.com/eating-smart/nutrition-101/i-cut-out-alcohol-heres-what-i-learned).**

The role of ethanol in alcoholic beverages can be considered to be rather similar to that of nicotine in tobacco, when it comes to causing cancer. Alcohol (ethanol) act as both local and systemic risk factors. It increases the permeability of oral mucosa, dissolve lipid components of epithelium, causes epithelial atrophy and interfere with DNA synthesis and repair, it also has genotoxicity and mutagenic effects, causing decreased salivary flow, affects the liver's ability to deal with toxic or potentially carcinogenic compounds, and their chronic use is associated with an impairment of innate and acquired immunity, resulting in increased susceptibility to infections and neoplasms[9,13].

## Possible Mechanisms of Action of Alcohol on the Oral Mucosa

In certain studies, with quantitative exfoliative cytology, the smears taken from normal buccal mucosa of alcohol dependent patients were compared with those obtained for age- and sex-matched control subjects. Regardless of age, increased alcohol use resulted in a statistically significant decrease in mean cytoplasmic area[13]. Malignant disease is associated with a decrease in cytoplasmic area and aberrant DNA profiles when exfoliative cytology is used[13]. However, a diploid profile was discovered when DNA profiles of buccal cells collected from alcohol-dependent individuals were examined using Feulgan-stained cells[13]. Furthermore, while polyploid DNA profiles were only identified in data from established oral malignancies, clinically normal mucosa may show more modest alterations in DNA and nuclear morphologic features. These might be just as essential as polyploidy in detecting future cancer in a patient who is predisposed to it. The micronuclear test is one tool that can identify small alterations. For many years, the micronucleus (MN) test, which measures tiny extranuclear material generated by the exclusion of chromosomal fragments, has been employed as a genotoxic exposure indicator. In the example where MN frequency was raised in buccal cells acquired from persons with a history of smoking and alcohol intake, the test was applied to smears obtained from alcohol users[13]. However, in another study were patients with oral cancer when assessed, they did not find any relation between daily alcohol intake and MN rates[13]. As cigarette usage was linked to higher MN, it's probable that even minor changes in alcohol-induced MNs would have been obscured by confounding factors[13].




**Flowchart 1: Mechanism of alcohol on oral mucosa (courtesy to Hye-Kyung Na et al, Molecular Basis of Alcohol-Related Gastric and Colon Cancer Int. J. Mol. Sci. 2017, 18(6), 1116; https://doi.org/10.3390/ijms18061116).**

More recent research found that it was impossible to distinguish between non-smokers and smokers who abused alcohol[13]. Other things to look at include the effects of alcohol kind, quantity, and years of exposure, as well as if nutritional or haematologic deficits have an impact on such outcomes. These issues must be solved in order to determine the MN assay's reliability in diagnosing oral cancer risk.[13]

## Mucosal Transport: Intercellular Passage

According to several studies on the permeability of the oral mucosa, ethanol may increase the penetration of carcinogens through the mucosa[13]. This might be due to intercellular transit of carcinogens into the oral mucosa or an increase in oral mucosa permeability. It has been proposed that more dilute ethanol (15%) is more effective than greater concentrations of ethanol because the latter acts as a chemical fixative, lowering permeability[13]. In another study, it was discovered that ethanol at a concentration of 50%, rather than 5% or 40%, enhanced the permeability of porcine oral mucosa to tritiated water and promoted the passage of bigger molecules like albumin[13]. However, lipid content remained constant at 15%, leading to assumption that enhanced permeability was caused by molecular rearrangement. The amount of ethanol in the system isn't the only thing that might affect permeability. Some studies have found that the risk of mouth cancer is equal

for spirits (40%) and beer (5%)[13]. Porcine tissue or tissue acquired from a human post-mortem has been employed in the majority of investigations. Furthermore, the fact that the results have been inconsistent supports the idea that more research using fresh human tissue should be done[13].

### Mucosal Transport: Intracellular Mechanisms

There are two types of membrane transport pathways: molecular and micromolecular. The latter can be further subdivided into endocytic and exocytic events. Pinocytosis (uptake of molecules smaller than 1 mm) and phagocytosis (uptake of molecules greater than 1 mm) are two types of endocytosis. Pinocytosis may be receptor mediated or fluid phase. Assessment of fluid phase endocytosis within buccal mucosal cells of alcoholics has been reported in few research[13]. Wherein Buccal smears were taken from 135 patients. For 1 hour at 37 °C, cell suspensions were cultured in Hams F10 culture media with bovine serum albumin–coated, fluorescent-labelled latex microspheres (0.02 mm in diameter). Confocal microscopy was used to validate microsphere uptake, and flow cytometry was used to assess endocytosed fluorescence levels. Endocytosis was dramatically decreased in individuals with detrimental alcohol consumption when compared to control participants. With endocytosis, there was no dosage response curve for units of alcohol ingested or cigarettes smoked. Endocytosis capacity did not improve after a 9–14 day period of apparent abstinence from alcohol[13].

## Genetic Susceptibility

A history of tobacco and alcohol use is the most common risk factor for SCCHN. Because only a small percentage of smokers and drinkers develop cancer, genetic predisposition may play an equivalent role in the aetiology of SCCHN. Large family studies have shown a three- to eightfold greater incidence of SCCHN in first-degree relatives of SCCHN patients, indicating that SCCHN has a genetic component[14].

In SCCHN patients, there is molecular epidemiologic evidence of genetic predisposition. Several case-control phenotypic and genotypic investigations are beginning to support the idea that genetic predisposition plays a role in the genesis of SCCHN[10]. Inherited variations in the efficacy of carcinogen metabolizing systems, DNA repair systems, or cell-cycle control/apoptosis systems, according to this concept, increase a person's risk of tobacco-induced malignancies. By using biomarker tests to identify such at-risk persons in the broader community, primary prevention, early detection, and secondary preventive efforts might be significantly improved[10].

Oral squamous cell carcinoma has been linked to a variety of genetic changes (SCC). Human chromosomes 1, 3, 5, 7, 8, 9, 13, 17, 18, 21, and 22 are often changed in oral SCC, according to cytogenetic and molecular research[15]. The role of fragile sites (FS) on human chromosomes in cancer risk and their implications have been widely debated in recent years. FS on chromosomes corresponds to oncogene or translocation locus in tumour cells. There are no reports of FS research on patients with oral cancer. The goal of this study was to see how important FS expression was in oral cancer patients before treatment and in healthy controls[15].

If the breakage loci are distributed non-randomly, chromosome lesions may be directly connected to cancer. The chromosome 5 was the most impacted, with four locations (5q31, 5q21, 5q14, and 5p14) expressing with a high frequency[15]. A mutagen-sensitive site that might be part of an oncogene region could be one of the breakpoints in translocations and inversions. A mutagen-sensitive site might also be involved in the second breakpoint, which could represent a regulatory area of a gene that normally codes for a protein important to a certain cell type at a specific stage of development. Such areas may aid in the transformation of an oncogene. At 5q34 on chromosome 5, there is a cellular oncogene called fms. There are other molecular studies supporting the results of this study[15]:

1. Allelic imbalance on the long arm of chromosome 5 has been implicated in oral SCC.
2. Loss of heterozygosity at 5q21q22 is reported to be common in oral SCC and correlated with advanced disease. It is also critical to elucidate the molecular structure of FS on chromosome 5 and study a possible relationship with tumorigenesis[15].

## Infectious Agents

Approximately 15% of oral cancer patients have no known risk factors, and the tumor may progress more rapidly in this group. This can be attributed to infections with viruses[Human Papilloma Viruses (HPV) and Epstein Barr Virus (EBV)], fungi like Candida albicans[8,10,14]. The role of viruses in carcinogenesis (by abrogation of p53 and pRb tumor suppressor genes and other cellular proteins involved, with subsequent alteration in the host genome function) is well documented with convincing evidences[16]. Chronic hyperplastic candidiasis, a rare oral fungal infection, is associated with the invasion of candidal hyphae into the oral epithelium and known to cause dysplastic changes leading to oral cancers[16].

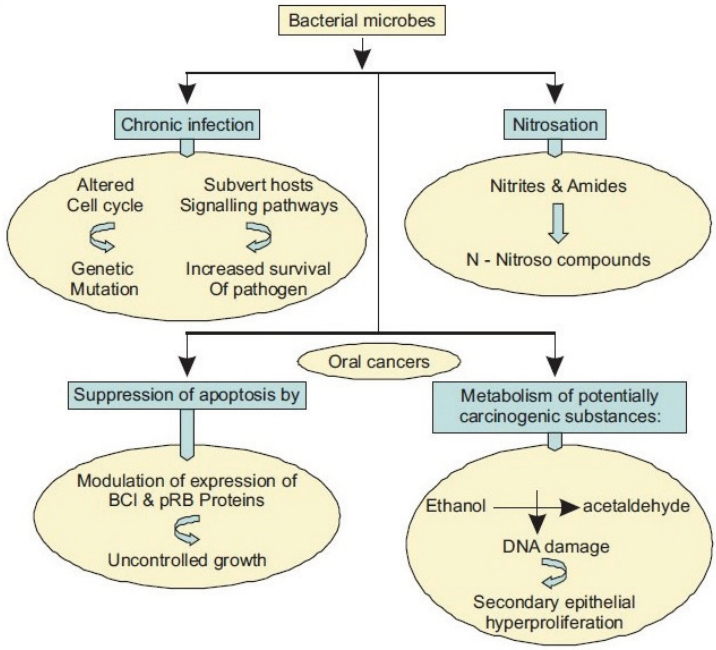
## Role of Bacteria

Several bacteria have been proven to induce persistent infections or create toxins that disrupt the cell cycle and cause abnormal cell growth. Chronic infections boost cell proliferation and DNA replication by activating the mitogen activated kinase (MAPK) and cyclin D1 pathways, which promote cell transformation and tumour growth. They also increase the rate of genetic mutation, which increases the incidence of cell transformation and the pace of tumour growth[16].

Several diseases produce pathogen accumulation inside cells, suppressing apoptosis mostly by regulation of Bcl-2 family proteins expression or inactivation of the retinoblastoma protein, pRb. This method creates a niche in which the intracellular virus may thrive despite the host immune system's attempts to kill infected cells through apoptosis. As a result, partially transformed cells are able to evade the self-destructive process and advance to a greater stage of transformation, eventually becoming tumorigenic[16].

Many pathogenic bacteria with intracellular access that cause persistent infection disrupt host cell signalling pathways, allowing the pathogen to thrive. The growth or suppression of tumour formation is dependent on the control of these signalling factors. Such infections can resemble some of the gross features observed in tumorigenesis, and the precancerous lesion that develops as a result of such infections can recur with antibiotic therapy and bacterial clearance[16].

Another method is the bacteria's metabolism of possibly carcinogenic chemicals. This is particularly the case in the oral cavity, where pre-existing local bacteria may contribute to cancer by converting ethanol to its carcinogenic product, acetaldehyde, at levels capable of inducing DNA damage, mutagenesis, and secondary epithelial hyperproliferation. Microbial carcinogenesis may also include nitrosation, which occurs when microbial cells catalyse the synthesis of N-nitroso compounds from nitrite and amines, amides, or other nitrosatable chemicals. Several bacteria species, including Escherichia coli, have strains capable of catalysing nitrosation. Yeasts and fungi can also contain nitrosating organisms. This specific nitrosamine looks to be a possible source of cancer, not just in the oesophagus but also in other mucosal sites such as the oral cavity[16].




**Flowchart 2: Various proposed paradigms for bacterial role in carcinogenesis. (Courtesy to Chocolatewala, et al, The role of bacteria in oral cancer, Indian Journal of Medical and Paediatric Oncology; 2010, Vol 31, Issue 4).**

It is critical to identify the organisms that predominate in these tumour tissues in order to determine the involvement of bacteria in the progression of oral cancers. This change in the oral microbiota in patients of oral squamous cell cancer is particularly intriguing. Research of intraoral carcinomas revealed a change in the microflora of the tumour tissue compared to the control locations, demonstrating this. Results of these researches was in agreement that the microenvironment of solid tumors is typically hypoxic with low pH, thus favoring the survival of only acid tolerant bacteria[16].

Another study conducted on the biofilms present on the surface of the oral SCC in patients also suggested an increase of the *Veillonella, Fusobacterium, Prevotella, Porphyromonas, Actinomyces* and *Clostridium* (anaerobes), and *Hemophilus*, Enterobacteriaceae and *Streptococcus* spp. (aerobes)[17].

Because of its relationship with tumours and propensity to create inflammation, the utilisation of Streptococcus anginosus in the carcinogenesis process is of

special interest. The oral bacteria S. anginosus has been linked to the tissues of esophageal, gastric, and pharyngeal cancers. S. anginosus DNA was shown to be 10 times more prevalent in esophageal cancer tissues than in oral cancer tissues, according to a study that used a highly specific quantification method of real-time polymerase chain reaction (PCR) for S. anginosus DNA[16].

## Role of Virus

Although several infectious agents have been hypothesised to have a role in head and neck carcinogenesis, current scientific data suggests that Epstein-Barr virus (EBV) and human papillomavirus (HPV) are the most typically identified as etiologic agents in head and neck carcinogenesis. EBV infection appears to be associated to the majority of nasopharyngeal carcinomas. Although HPV (mainly type 16) infection is linked to around half of all oropharyngeal carcinomas, HPV does not appear to have a significant role in OCC. Infection with HPV may potentially have a role in the development of squamous cell carcinomas in the sinonasal tract. Although infection with the herpes simplex virus has been mentioned as a risk factor for OCC, this link has not been proven[10].

### *1. Human papilloma viruses*

When cytopathic effects of HPV (koilocytosis) were seen on light microscopy of oral carcinomas and the presence of HPV antigens in 40 oral carcinomas were investigated using immunohistochemistry, Syrjanen et al. were the first to present data on HPV as an etiological factor in OSCC in 1983[18].

Chang et al. analysed these biopsies for the presence of HPV DNA using in situ hybridization (ISH) and polymerase chain reaction (PCR) a few years later in 1990, and 12 of 40 revealed the presence of HPV 11, 16, or 18 DNA[19].

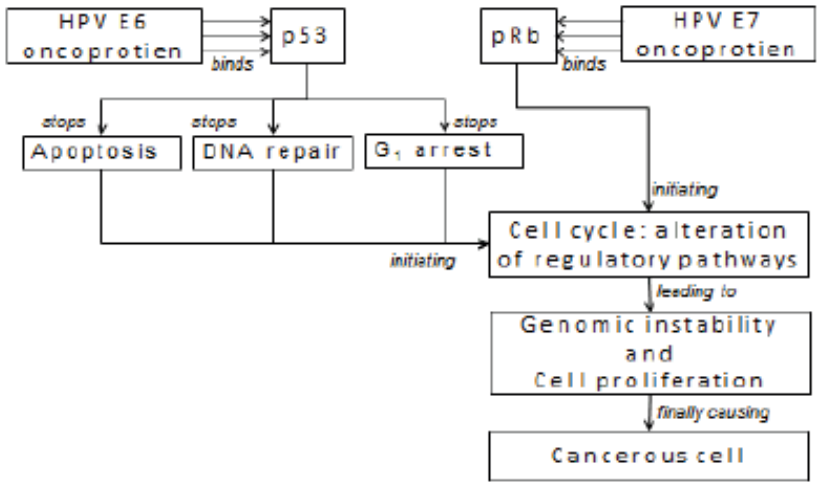
Several studies have shown the presence of HPV in various quantities in oral or oro-pharyngeal squamous cell carcinoma sites other than the uterine cervix, particularly those genotypes with recognised high oncogenic potential. It also suggests that HPV evidence in mucosal tissue of the head and neck other than the oral cavity is much easier to detect. The reduced detection rate of HPV may be due, at least in part, to the oral cavity's motility, as well as saliva secretion and cleansing. HPV testing is recommended in healthy individuals' normal mucosa as compared to its identification in malignant tumours. As a result, standardisation of sample collection and analysis procedures is required in order to get reliable data and compare the results of different investigations[20].

HPV DNA has been found in both the primary tumour and its lymph node metastases in five studies. The study also discovered that when cigarette smoking

decreased, the rate of HPV-negative malignancies decreased by 50%. Overall, males were at the higher risk, and the data imply that it may be related to an increase in oral sex. HPV is most known for causing cervical cancer in women, although because of improved screening, the frequency of such tumours has reduced over time. Individuals with HPV-positive oral malignancies have a higher survival rate than those with cancer caused by other factors, presumably because their tumours produce less genetic damage, making them more responsive to treatment[20].

After 20 years of active study on the issue, Syrjanen et al stated in 2005 that their findings from the early 1980s, that HPV appears to be connected with at least a fraction of oral carcinomas, are still relevant. Sisk et al. found no significant difference in HPV incidence between younger and older individuals, indicating that HPV plays a comparable role in all age groups[20].

Although it has been proposed that high-risk HPV infection may cause some percentage of oral carcinogenesis, additional evidence is now emerging suggesting HPV infection has the capacity to profoundly modify the proliferative phenotypes and outcomes of oral cancer. As a result, evaluating the ability of HPV to modify phenotypic characteristics of previously transformed oral carcinomas has become a critical step in better predicting prognosis and treatment choices for individuals with oral cancer[20].




**Flowchart 3: Role of HPV in oral cancer (courtesy to Gupta AA (2015) Unconventional Causes of Conventional Oral Cancer. J Clin Exp Pathol 5:254. doi: 10.4172/2161-0681.1000254).**

### 2. *Herpes simplex virus*

According to certain research, HSV can change cells by functioning as a mutagen. Previous research found chromosomal abnormalities in HSV-infected cells, which are indicative of mutagen effects. Studies of the occurrence of mutations at the hypoxanthine guanine ribosyl transferase locus of mammalian cell genomic DNA found 2–4 times increase in mutation frequency following infection by HSV 1 that had been inactivated by UV radiation. Pilon et al., discovered the same behaviour in cells infected with HSV2[20].

Steele and Shillitoe proposed in 1991 that HSV 1 expresses a viral protein known as MUT. Other research has linked MUT to the p40 protein family, which is found in the nuclei of infected cells, although the function of this protein family has yet to be determined. Steele and Shillitoe postulated that the p40 family or the MUT protein may be involved in host cell shutoff based on these findings. Mutant DNA mismatch repair proteins recognise and repair erroneous insertions, deletions, and mis-incorporation of bases that occur during DNA replication and recombination. Mutations in this gene cause genetic abnormalities called microsatellite instability, which has been linked to cancer. Rearranged chromosomal DNA was found in certain HSV 1 produced mutant plasmids, which is characteristic of malignancies linked with DNA tumour viruses. As a result, it was established that HSV-induced chromosomal rearrangements may be present in malignancies[20].

### 3. *Epstein-Barr virus*

Keisuke et al. conducted research to see if EBV was present in different squamous cell proliferative lesions in the mouth. For determining the presence of EBV DNA and EBV encoded short messenger RNA, they used PCR and in situ hybridization. EBV genome positivity was found in about 60% of SCC, however no EBV genome positivity was found in papilloma. The oral hairy leukoplakia lesion found in AIDS patients, on the other hand, has been proven to be EBV-related. EBV viral infection of the oral squamous epithelium may be carcinogenic, or the virus may only exist in epithelial cells of squamous cell carcinoma, carcinoma in situ, and leukoplakia, according to Keisuke et al. González-Moles et al. discovered a link between different grades of OSCC and EBV DNA positivity, as well as the fact that the percentage positivity of EBV DNA rises as the OSCC progresses from highly differentiated to poorly differentiated[20].

### 4. *Hepatitis C virus*

Hepatitis C virus (HCV), an RNA virus, can be detected easily in the saliva of patients with chronic liver disease and is the cause of non-A, non-B hepatitis, liver

cirrhosis, and hepatocellular carcinoma in the vast majority of cases. Johnson et al. hypothesised that HCV may have a role in illnesses other than liver disease, and concluded that because the oral cavity is regularly exposed to HCV viruses, the risk of genetic instability in these cells is increased[21].

Nagoa et al. investigated a sample of 100 patients, with 88 of them having SCC. Anti-HCV antibodies were found in 25 patients' serum. The precise mechanism is unknown. Anti-HCV antibodies were detected in significant abundance in individuals with oral lichen planus, according to Gandolfo S et al (OLP). Nagao et al. hypothesised that because OLP is a squamous cell disease, the squamous cells of the oral area are constantly exposed to HCV through saliva as well as serum in HCV-positive patients, and that this may play a role in the development of SCC and OLP in these individuals[20,22].

## Dental Factors

Oral cancer can be caused by a variety of factors, including poor oral hygiene, damage from sharp teeth, and ill-fitting entures. In the setting of a field of molecular change and the continued presence of local carcinogens, such trauma may pinpoint the spot at which malignant transformation occurs. In a well cited research from Beijing published in 1990, the number of lost teeth and the use of metal dentures were linked to oral cancer regardless of other risk factors. There's also a link between bad periodontal health and mouth cancer[14].

## Diet

Diets heavy in animal fats and low in fruits and vegetables, according to epidemiologic evidence from classic case-control studies, may increase the incidence of SCCHN. McLaughlin et al found an inverse relationship between fruit intake and the incidence of oral and pharyngeal cancer in a sample of 871 people with oral and pharyngeal cancer and 979 cancer-free controls[10]. More recently, after accounting for smoking and alcohol use, European and American research have supported the preventive benefits of fruits and vegetables as well as the danger of animal fat consumption. Consumption of salted fish has been linked to an elevated risk of nasopharyngeal carcinoma in several case-control studies, possibly due to the high nitrosamine content in preserved foods like salted fish. Poor diet appears to be second only to tobacco as a cause of oral malignancies globally[10].

There is also some evidence that vitamin A and beta-carotene are crucial for the preventive benefit of a diet rich in fruits and vegetables, and that carotenoids deficits

are a risk factor for SCCHN and lung malignancies. Others have discovered that a high vitamin C and E consumption is also beneficial[14]. Vitamin A and associated carotenoids (especially beta carotene), vitamins C and E, and selenium appear to be particularly protective against most epithelial malignancies, with antioxidant activity accounting for much of the impact. Antioxidants work by decreasing free radical reactions that can lead to DNA mutations and alterations in cellular membrane lipid peroxidation. Modulation of carcinogen metabolism, maintenance of optimal cell differentiation, reduction of cell proliferation and oncogene expression, preservation of immunological function, and prevention of synthesis of endogenous carcinogens are all protective functions of micronutrients[14].

Folate is also a powerful preventive dietary component, according to the International Head and Neck Cancer Epidemiology Consortium's thorough meta-analyses (INHANCE). Tea, particularly green tea, which contains high quantities of polyphenols, is currently being studied for its potential health benefits. These are potent antioxidants that can stop carcinogenesis from starting and progressing. Nutritional care is crucial in the treatment of HNSCC patients[14].

## Radiation

There has been no conclusive evidence of a link between ionising radiation exposure and the development of SCCHN. Long-term exposure to sunlight, on the other hand, is a significant risk factor for the development of SCC of the lip in those with fair complexions and those who work outside. Thyroid tumours, head and neck sarcomas, and salivary gland cancers, particularly paranasal sinus cancers, have all been linked to gamma irradiation. Therapeutic irradiation of head and neck cancers does not appear to cause secondary and primary squamous carcinomas of the aerodigestive tract, although it is linked to a higher incidence of head and neck sarcoma. Children who have had therapeutic irradiation are especially vulnerable. Furthermore, irradiation of the head and neck for environmental, medical diagnostic, and therapeutic purposes is all linked to salivary gland cancer. Mucoepidermoid carcinomas appear to be the most prevalent radiation-induced malignancy, according to these investigations, which indicated a substantial dose–response connection with risk increasing with dosage[10,14].

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# Chapter 3

# Classification of Oral Cancer

*Dr. Karthikeya Patil*

***Keywords: Carcinoma, Classification, Odontogenic, Sarcoma, Lymphoma.***

To facilitate communication between health-care providers, a classification system was established by the World Health Organization (WHO). The classification system is updated from time to time based on advances in technology and outcome data. According to the WHO classification of tumors, the morphology of the cells and the tissue architecture as seen in light microscopy is used to define the neoplasm, which may correlate with the biology and behaviour of the cancer[23].

## WHO Classification of Oral Cancer[23]:

1. Epithelial cancer
    i. Squamous cell carcinoma
        a. Verrucous carcinoma
        b. Basaloid squamous cell carcinoma
        c. Papillary squamous cell carcinoma
        d. Spindle cell carcinoma (sarcomatoid SCC)
        e. Acantholytic squamous cell carcinoma
        f. Adenosquamous carcinoma
        g. Carcinoma cuniculatum
    ii. Lymphoepithelial carcinoma
        a. Salivary gland cancer
        b. Salivary gland carcinoma
        c. Acinic cell carcinoma
        d. Mucoepidermoid carcinoma

e. Adenoid cystic carcinoma
f. Polymorphous low-grade adenocarcinoma
g. Basal cell adenocarcinoma
h. Epithelial-myoepithelial carcinoma
i. Clear cell carcinoma, NOS
j. Cystadenocarcinoma
k. Mucinous adenocarcinoma
l. Oncocytic carcinoma
m. Salivary duct carcinoma
n. Myoepithelial carcinoma
o. Carcinoma ex pleomorphic adenoma

iii. Salivary gland adenomas

2. Soft tissue cancer
   i. Kaposi sarcoma
3. Hematolymphoid cancer
   i. Diffuse large B-cell lymphoma
   ii. Mantle cell lymphoma
   iii. Follicular lymphoma
   iv. Extranodal marginal zone B-cell lymphoma of MALT type
   v. Burkitt lymphoma
   vi. T-cell lymphoma
   vii. Extramedullary plasmacytoma
   viii. Langerhans cell hystiocytosis
   ix. Extramedullary myeloid sarcoma
   x. Follicular dendritic cell sarcoma
   xi. Secondary tumors

## WHO Classification of Odontogenic Cancer[23]:

1. Odontogenic carcinomas
    i. Metastasizing (malignant) ameloblastoma
    ii. Ameloblastic carcinoma—primary type
    iii. Ameloblastic carcinoma—secondary type (dedifferentiated), intraosseous
    iv. Ameloblastic carcinoma—secondary type (dedifferentiated), peripheral
    v. Primary intraosseous squamous cell carcinoma—solid type
    vi. Primary intraosseous squamous cell carcinoma derived from keratocystic odontogenic tumor
    vii. Primary intraosseous squamous cell carcinoma derived from odontogenic cysts
    viii. Clear cell odontogenic carcinoma
    ix. Ghost cell odontogenic carcinoma
2. Odontogenic sarcomas
    i. Ameloblastoma fibrosarcoma
    ii. Ameloblastic fibrodentino- and fibro-odontosarcoma
3. Other tumors
    i. Melanotic neuroectodermal tumor of infancy

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# Chapter 4

## Clinical Presentation of Oral Cancer

*Dr. Mahesh K P, Dr. Aparna*

***Keywords: Manifestation, Leukoplakia, Erythroleukoplakia, Erythroplakia, Ulceration***

In late stages of OSSC, the clinical presentation is so distinctive that there is frequently a strong suspicion of malignancy. In contrast, it is very feasible to make an incorrect diagnosis early on. Because clinical criteria alone are inadequate, a biopsy and histological analysis are always required to confirm the diagnosis[24].

A clinician should always conduct a complete clinical examination of the oral mucosa, focusing on those areas that are more vulnerable, such as the lateral borders of tongue and the floor of mouth. Palpate the cervical lymph nodes in conjunction with the oral cavity to search for any masses in the neck that might be metastases. The size of OSCC lesions varies, ranging from a few millimetres to several centimetres in the most severe instances. Since the initial lesions are small, they are frequently asymptomatic[24].

OSCC can manifest clinically in various forms. Any solitary oral mass, ulcer, white or red lesion persisting for more than 3 weeks, non-healing socket, numbness, or unexplained loose tooth should be considered cancer unless proven otherwise[25]. Features suggestive of OSCC include:

1. Red lesion (erythroplasia or erythroplakia).
2. Mixed red/white lesion (erythroleukoplakia); irregular white lesion (verrucous leukoplakia).
3. Pain or numbness.
4. Ulcer with fissuring or raised exophytic margins.
5. Lump/mass.
6. Extraction socket not healing.
7. Loose tooth.
8. Abnormal blood vessels supplying a lump.[25]

Every clinician's objective should be to detect OSCC and possibly malignant tumours as early as possible. Various diagnostic tools, such as vital staining (with toluidine blue) and optical methods to increase visibility, cytology, molecular markers in tissues, and even saliva, are being used in this regard. It's critical to treat potentially malignant and malignant lesions as soon as possible, with the awareness that what's seen clinically is nearly often only a small fraction of the true lesion[25].

## Initial Stages

It's critical to diagnose OSCC early, and it should be suspected in individuals who have solitary oral lesions that last longer than 3 weeks.

The erytholeukoplastic lesion is the most common clinical manifestation of these early malignant lesions. It is well-defined and comprises of red or red and white regions with a slight roughness. On palpation, the soft tissue's elasticity transforms to a harder feeling ("induration"). Although there is rarely any pain, there may be some discomfort[24].

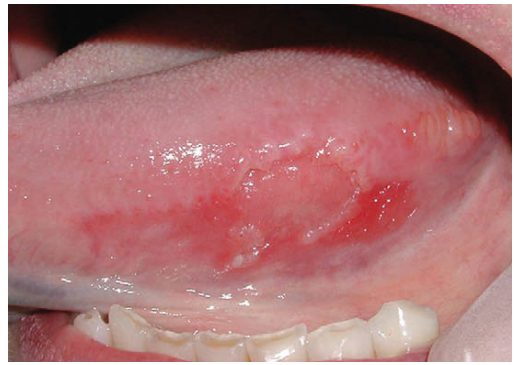

**Fig. 3: Carcinoma on the left lateral border of the tongue (image courtesy to Bagan J et al, Review Oral cancer: Clinical features, Oral Oncology, 46 (2010) 414–417).**

## Advanced Stage

Ulceration, lymph node involvement, and fixation to underlying tissues are all common features of oral cancer. One of the most prevalent and well-known features of OSCC is ulceration. On palpation, the ulceration has an irregular floor and margins, as well as being raised and firm. When a lesion is big, the patient frequently has intense pain that radiates from the lesion to the ipsilateral side[24].

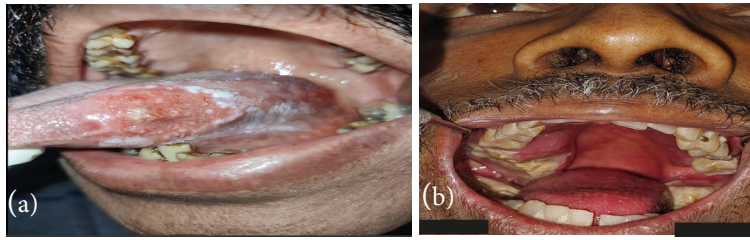


**Fig. 4: Oral squamous cell carcinoma (a) on the left lateral border of tongue (b) right alveolar ridge (image courtesy: clinical photograph collected from Department of Oral Medicine and Radiology, JSS Dental College and Hospital, JSSAHER, Mysuru.)**

Exophytic tumours with warty surfaces, poorly defined borders, and hard on palpation may be detected in these advanced stages[24]. Some OSCC patients have chin paraesthesia or numbness. Others show up as a mass with abnormal supplying blood vessels, dysphagia, weight loss or delayed healing after a tooth extraction. These advanced instances may be linked with neck metastases, which manifest as cervical lymph node enlargement, particularly if a lymph node is hardened or fixed. When the likely site for the primary lesion is the tongue base, tonsil, or nasopharynx, a cervical lymph node enlargement is found in the lack of any visible primary tumour (approximately 5% of the time)[26].

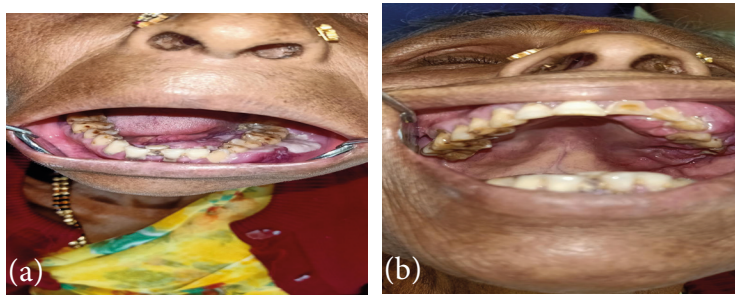


**Fig. 5: (a) Ulcerated tumor in the floor of the mouth extending to involve the gingiva and buccal vestibule (b) Tumor of left maxilla (image courtesy: clinical photograph collected from Department of Oral Medicine and Radiology, JSS Dental College and Hospital, JSSAHER, Mysuru.)**

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# Chapter 5

# Staging of Oral Cancer

*Dr. Mahesh K P, Dr. Karthikeya Patil*

***Keywords: Tumor, Lymph Node, Metastasis, Invasion, Carcinoma***

Cancer staging determines the degree of the illness in terms of anatomic spread and is critical for the establishing evidence-based treatment options. The current staging system proposed by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (IUAC) includes defining prognosis and evaluating different treatment methods (Union Internationale Contre le Cancer, UICC). The AJCC and UICC's staging recommendations are the outcome of a 45 year development process[10].

The American Joint Committee for Cancer Staging and End-Results Reporting began this procedure in 1959 and completed in 1977 with the release of the Manual for Cancer Staging. The tumour, node, and metastasis (TNM) classification system was devised on the basis that anatomic extension (T) and spread to lymph nodes (N) and elsewhere (M) better described the life history of cancer, and a single TNM classification has been used globally since 1987. The idea was that more advanced stages would exhibit increasingly worse, hence this shorthand approach of identifying illness extent was also utilized to construct stage groups. The TNM system has gone through multiple revisions since its inception to accommodate for new breakthroughs in diagnostic evaluation and recently published therapy results[10].

## TNM Staging

**Table 1: Staging of oral squamous cell carcinoma[23]**

| **Staging of Oral Squamous Cell Carcinoma** | |
|---|---|
| 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 |
| T4a | Moderately advanced local disease (lip) Tumor invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (chin, nose, oral cavity)<br>Tumor invades through cortical bone, into deep/ extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face. |
| T4b | Very advanced local disease (lip and oral cavity) Tumor invades masticator space, pterygoid plates, or skull base, or encases internal carotid artery |
| 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.<br>- In multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension.<br>- 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 |
| Mx | Distant metastasis cannot be assessed |
| M0 | No distant metastasis |
| M1 | Distant metastasis |

T1, T2, and T3 lesions of the oral cavity are differentiated by two-centimetre increments in initial tumour size. On the other hand, tumour invasion patterns are employed to classify an oral cavity tumour as a T4a lesion. T4a lesions are tumours that penetrate through cortical bone into the deep (extrinsic) muscle of the tongue, the maxillary sinus, or the skin of the face. Surface cortical bone erosion alone is not enough to identify a tumour as T4. The criteria for assigning a T4a

classification to tumours developing from other locations within the oral cavity differ slightly from those used to assign a T4a diagnosis to lip cancer[10]. The inclusion of T4b, which indicates the presence of unresectable disease by the sixth edition of the AJCC Cancer Staging Manual has changed the staging of oral cavity cancer. Unresectable tumours include those that invade the masticator space, the pterygoid plates, or the skull base, as well as those that enclose the internal carotid artery. With the exception of the nasopharynx, the parameters used to assign a value to N for the amount of regional lymph node metastases from oral cancer are identical to those used for the larynx and pharynx. A single ipsilateral lymph node with a greatest dimension of 3 cm or less is classed as N1, whereas a single ipsilateral lymph node with a greatest dimension of more than 3 cm or the presence of numerous lymph node metastases is classified as N2. N3 classification is given to lymph nodes that are larger than 6 cm in diameter. M0 refers to the lack of metastatic illness, whereas M1 refers to the existence of distant metastases. To categorise the tumour, the T, N, and M values are added together. cTNM, or simply TNM, is the abbreviation for clinical staging[10].

Each potential combination of T, N, and M values is assigned to one of multiple TNM stage groupings under the AJCC categorization method. The stage groupings are meant to make each stage group "relatively homogeneous in terms of survival" so that "the survival rates of these stage groupings for each cancer site are distinct," and to provide a mechanism for comparing similar groups of patients who might be candidates for different therapeutic options[10].

**Table 2: Stage grouping[23]**

| **Stage grouping** | |
|---|---|
| Stage 0 | Tis N0 M0 |
| Stage I | T1 N0 M0 |
| Stage II | T2 N0 M0 |
| Stage III | T3 N0 M0 |
| | T1 N1 M0 |
| | T2 N1 M0 |
| | T3 N1 M0 |

The stage III category includes early primary tumours with a single lymph node metastasis (N1) and cT3N0M0 cancers. T4 lesions are classified as T4a

(resectable) or T4b (unresectable), and stage IV is divided into stages IVA, IVB, and IVC. Patients with T4a primary tumours or N2 regional nodal illness are classified as stage IVA, whereas those with T4b or N3 status are classified as stage IVB. The existence of distant metastases is indicated by the stage IVC grouping (M1)[10].

TNM is given prefixes or suffixes to offer more descriptive information so that these situations may be evaluated independently. A lower-case p precedes the TNM classification to indicate pathologic staging (pTNM). It's important to remember that pathologic T classification might be an underestimate of true tumour size since mucosal margins can shrink by up to 30% following resection. The retreatment classification (rTNM) is used when further treatment for recurrent cancer is expected following a disease-free period. If the stage is determined at autopsy for cancer that was not apparent previous to death, the prefix "a" is used (aTNM). The m suffix, which is written parenthetically after the T characteristic[pT(m)NM], denotes multiple primary tumours at a single location. To show staging during or after multimodality therapy, the y prefix should appear before the c or p. (ycTNM or ypTNM). This prefix should only be used when a choice on further surgical therapy is being considered after multimodality therapy has been completed. This choice is usually taken within two months of the conclusion of multimodality therapy[10,23].

These staging principles are applicable to all types of cancer. Sarcomas, lymphomas, and melanomas should all be staged according to their own set of rules. The tumour staging form in the sixth edition of the AJCC Cancer Staging Manual includes histologic grade, which is a qualitative judgement that is not utilised to stage oral cancer. The histologic grade of squamous cell carcinoma is subjectively classified as well, moderately, or poorly differentiated carcinoma based on its resemblance to normal mucosa[10].

The existence of residual tumour should be indicated using the following designations after resection: presence of residual tumour cannot be identified (RX), no residual tumour (R0), microscopic residual tumour (R1), and macroscopic residual tumour (R2) (R2). Descriptors that indicate the presence of lymphatic and venous invasion can also be added to the stage form. It's also a good idea to keep track of any perineural invasion[10].

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# Chapter 6

# Diagnostic Aids in Early Detection of Oral Cancer

*Dr. Mahesh, Dr. Karthikeya Patil And Dr. Aparna V*

***Keywords: Toluidine blue, Chemiluminescence, Biopsy, Thernostics, Biomarkers***

Oral cancer detection at a late stage leads to high mortality and morbidity. The treatment and survival rate depends on the time of diagnosis. Early diagnosis for detecting these lesions and predicting their progress is very important to provide care to patients and improve their quality of life and survival rate[3].

**The characteristics of a good screening test includes[2]**

1. It should be simple, safe and acceptable to the public.
2. To detect disease early in its natural history.
3. To detect those lesions which are likely to progress into malignancy.
4. To detect lesions which are treatable or where an intervention will prevent progression.
5. Should have a high positive predictive value and low false negatives (high sensitivity).

**The commonly used diagnostic aids of oral cancer is as follows[4,6]:**

1. Non invasive techniques
    i. Clinical examination
    ii. Toluidine blue and Lugol's Iodine staining
    iii. Saliva-Based Oral Cancer Diagnosis
        a. Genomic substances
        b. Transcriptomic substances
        c. Proteomic substances

2. Light-based detection systems:
    i. Chemiluminescence (ViziLite Plus; Microlux/DL,Orascoptic-DK)
    ii. Tissue fluorescence imaging (VELscope)
3. Advanced diagnostic aids
    i. Optical Biopsy:
        a. Tissue fluorescence spectroscopy
        b. Raman spectroscopy
        c. Elastic scattering Spectroscopy
        d. Differential path-length spectroscopy
        e. Nuclear magnetic resonance spectroscopy
        f. Confocal reflectance microscopy (CRM)
        g. Optical Coherence Tomography
        h. Angle-resolved low coherence interferometry (A/LCI)
    ii. Thernostics
    iii. Biomarkers
    iv. Laser capture microdissection
4. Invasive techniques
    i. Biopsy
        a. Surgical biopsy
        b. Fine needle aspiration cytology
        c. Exfoliative cytology
        d. Liquid based cytology
        e. Oral Brush biopsy
        f. Frozen sections

## Non-Invasive Techniques

### Clinical Examination

The procedure for identifying oral lesions is part of the overall process that includes obtaining a patient's personal, medical, and habit histories, as well as carrying out a

full clinical oral examination. Oral cancer screening has traditionally been done with a conventional oral examination (COE) under normal (incandescent) light. All mucous membranes and gingival surfaces should be thoroughly examined, with special attention paid to the lateral border of the tongue, the floor of the mouth, and the throat, which are all potential locations for oral cancer. The dentist should next perform a bimanual digital palpation to see whether there are any abnormal enlargements of the salivary glands or lymph nodes in the face or neck[27].

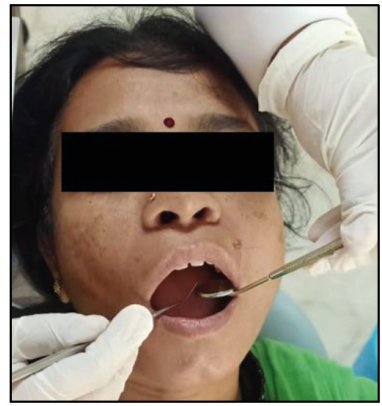


**Fig. 6: Clinical examination of the patient (image courtesy: clinical photograph collected from Department of Oral Medicine and Radiology, JSS Dental College and Hospital, JSSAHER, Mysuru.)**

Any sign or symptoms must be evaluated thoroughly. On intra oral examination, any evidence of mobility, tilting, or a sharp cusp of the teeth should be checked thoroughly. To complete the clinical assessment, a radiographic examination may be required. Any lesion must be palpated for induration or stiffness. Any mass or aberrant change in the mucosa must be palpated, even if it appears to be normal. Any signs of roughness, ulceration, redness, or induration must be further studied by histopathologic examination while examining an OPL[2].

Clinical examination can help to reduce the mortality rate of oral cancer. According to one study, oral examination can avoid over 40000 deaths worldwide by reducing OSCC mortality by 32% in high-risk adults. Even self-examination can help discover oral cancer earlier, however treatment adherence is still poor (32%). False positive referrals for definitive diagnosis can cause anxiety, which can be alleviated by providing public education to those who have oral examination[2].

Despite the benefits of clinical examination, it has been shown that between 4.5 and 15.3% of OPL and early-stage oral cancers cannot be identified adequately by visual inspection alone, and may be overlooked even by highly trained professionals, potentially increasing the false negative rate. Because of this inherent flaw in visual examination, different diagnostic tools can be used to enhance oral cancer early detection[2].

## Toluidine Blue and Lugol's Iodine Staining

Toluidine blue, commonly known as tolonium chloride, is an acidophilic meta chromatic dye that stains acidic tissue components preferentially, including DNA and RNA. In both water and alcohol, it is only partially soluble. As it binds to nucleic acids (DNA or RNA), it aids in improved imaging of high-risk locations, particularly in OSCC and premalignant lesions with fast cell proliferation. It stains mitochondrial DNA, cells with higher than normal DNA content, and dysplastic and malignant cells with altered DNA[4].

Hence, it has been used for many years as an aid to the identification of clinically occult mucosal abnormalities and as a useful way of demarcating the extent of a potentially malignant lesion prior to excision[28].

Toluidine blue staining will guide the clinician to

1. Detect carcinoma in situ and early invasive OSCC.
2. Surgical field delineation for biopsy sites.
3. Detection of secondary malignancies or satellite tumours.
4. Recognizing recurrence after therapy.[28]

Patients who are at risk of developing oral cancer or who have proven neoplasms in other areas of the aerodigestive tract may benefit from vital staining of the oral epithelium as a way of surveillance. TB has been utilised as a critical stain to highlight possibly malignant oral lesions and may reveal early lesions that would otherwise go undetected during a clinical examination. Furthermore, it can show the complete extent of dysplastic epithelium or carcinoma prior to excisions, detect multicentric or second tumours, and aid in the follow-up of oral cancer patients. It can be used to determine the extent of carcinoma control and to choose a biopsy sample location in premalignant lesions. Loss of heterozygosity may be detected in TB-stained lesions. TB-stained tissue may appear dark royal blue or pale royal blue color[28].

## Principle

TB stains tissues based on the principle of metachromasia. The dye reacts with the tissues to produce a color different from that of the original dye and from the rest of the tissue. Metachromasia is significant because it is very selective, with just a few tissue structures capable of staining metachromatically. It is a phenomenon in which a dye may absorb light at various wavelengths depending on its concentration and surroundings, and it can change colour without affecting its chemical structure. Dye aggregation is a particular, organised kind of dye aggregation that causes this colour shift. There must be free electronegative groups on the surface of tissues for metachromasia to develop[28].

## Procedure

For oral administration, TB is usually produced at a concentration of 1%. 1 gm of TB powder, 10 ml of 1% acetic acid, 4 ml of absolute alcohol, and 86 ml of distilled water make up 100 ml of 1% TB. The pH level is normally kept around 4.5. To eliminate debris, the procedure normally entails washing the mouth twice with water for 20 seconds each time. After that, a 20 second application of 1% acetic acid is used to eliminate the ropey saliva. This is followed by a 20 second application of % TB, either with a cotton swab if a mucosal lesion is present or as a rinse if no visible lesion is present. To lessen the degree of mechanically retained stain, 2 rinses with 1% acetic acid were conducted once again. Finally, water is used to rinse the mouth[28].

The interpretation is based on the color; a dark blue (royal or navy) stain is considered positive, light blue staining is doubtful and when no color is observed, it is interpreted as negative stain. Under normal conditions, nucleated scales covering the papillae on the dorsum of the tongue as well as the pores of seromucinous glands in hard palate are frequently stained with TB[28].

This acidophilic metachromatic nuclear stain helps to identify regions of carcinoma in situ or invasive cancer from normal tissue by applying a 1% aqueous TB solution for 30 seconds. A TB staining that is positive might look as a dark royal blue staining. For malignant lesions, it is very sensitive and somewhat specific. It has a lower sensitivity for premalignant lesions, with reports of up to 58% false negatives when detecting mild-to-moderate dysplasia. According to Rosenberg and Cretin, TB staining in oral cancer screening has a sensitivity of 93.5–97.8% and a specificity of 73.3–92.9 %[6].

Toluidine blue is currently best utilised as an adjuvant to clinical examination in the assessment of the biologic potential of possibly malignant oral lesions by experienced physicians. Toluidine blue stained visible lesions with high risk molecular patterns preferentially and predicted risk and prognosis in situations where there was little to no microscopic evidence of dysplasia, according to a recent study[1].

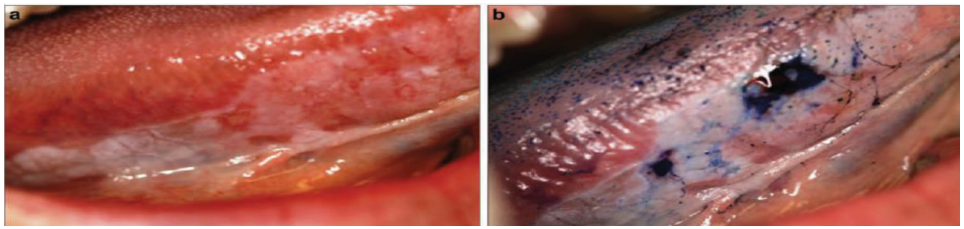



**Fig. 7: TB staining on a lesion in the right lateral border of the tongue (image courtesy to Messadi DV, Diagnostic aids for detection of oral precancerous conditions, International Journal of Oral Science (2013) 5, 59–65; doi:10.1038/ijos.2013.24).**

Early carcinomas and other oral lesions have been detected using a combination of Lugol's iodine and toluidine blue. The malignant alteration is delineated using Lugol's solution, which generates a brown black stain when the iodine reacts with the glycogen content. Toluidine blue and Lugol's iodine are important adjuncts in the diagnosis of high-risk patients and in the selection of a biopsy site for wide-field malignancies prior to therapy[4].

TB application is a significant adjunct to clinical examination since it may help identify locations that require biopsy and delineate borders of lesions, which can lead to a more prompt diagnosis, allowing for the benefits of earlier therapy and directing surgical care. Although the technique's specificity is lower, which could be due to the inability to biopsy normal tissues that do not take up TB, it can still be used as a diagnostic aid because false-positive results are less concerning in a clinical setting than false-negative results, and any positive findings should be confirmed on biopsy for the presence of dysplasia or carcinoma. In addition, any lesion that is not clinically suspicious but shows After 10–14 days, TB uptake should be re-evaluated to see if the situation has improved. If the lesion is still positive for tuberculosis, it should be regarded worrisome, and a biopsy should be conducted to rule out malignancy. The value of TB staining would be enhanced much more if staining procedures and interpretation were standardized[28].

## Saliva-Based Oral Cancer Diagnostics

Saliva from patients has been utilized in an innovative approach to develop molecular biomarkers for the identification of oral cancer. Saliva is a mirror of the body, reflecting nearly the whole range of normal and pathological states, and its use as a diagnostic fluid satisfies the desire for a low-cost, non-invasive, and easily available diagnostic tool. The discovery of analytes in the saliva of healthy and sick people points to saliva's potential as a local and systematic diagnostic tool[8].

Oral cancer detection based on saliva is a non-invasive alternative to blood investigations with an overall accuracy rate of roughly 85%. It is a useful tool for diagnosing oral cancer, evaluating prognosis, and tracking post-treatment outcomes[4]. The utility of salivary transcriptome diagnostics is helpful in the detection of oral cancer. In the saliva of head and neck cancer patients, promoter hypermethylation patterns of TSG p16, O6-methylguanine-DNA-methyltransferase, and death associated protein kinase were discovered, as well as high salivary counts of Capnocytophaga gingivalis, Prevotella melaninogenica, and Streptococcus mitis. Nowadays, saliva testing for genetic patterns which are linked with oral cancer is gaining interest for research. Failure to diagnose oral cancer at an early stage is one of the factors contributing to its high fatality rate. Saliva testing for oral cancer detection has shown to be a historical aim that must be achieved by the public in the future for better and quicker management of OSCC[4].

Relevant markers include oncogenes (e.g., C-myc, cFos, C-Jun), anti-oncogenes (e.g., p53, p16), cytokines (e.g., transforming growth factor-β1, interleukin (IL)-8, and -1β), growth factors (e.g., vascular endothelial growth factor, epidermal growth factor, and insulin like growth factor), extracellular matrix-degrading proteinases (MMP1, MMP2, MMP9), hypoxia markers (hypoxia-inducible factor-α, carbonic anhydrase-9), epithelial-mesenchymal transition markers (e.g., Ecadherin, N-cadherin, and β-catenin), epithelial tumour factors (Cyfra 21–1), cytokeratins (CK13, 14 and 16), microRNA molecules, and hypermethylation of cancer-related genes (p16 and death-associated protein kinase)[6].

### Genomic Substances

Point mutation, deletion, translocation, amplification, and methylation are markers in the form of alterations in the host DNA of dysplastic or cancer cells. Loss of heterozygosity on chromosomes 3p, 9q, 13q, and 17p is thought to be a precursor to mouth cancer. Allelic loss on chromosomes 3p and 9q raises the probability of malignant transformation by 3.8-fold in certain studies; the risk increases to 33-fold when loss of heterozygosity occurs on chromosomes 4q, 8p, 11q, 13q, and 17p

as well[6]. Exfoliated OSCC cells in saliva can also be detected using mitochondrial DNA mutations. Such mutations have been found in 46% of patients with head and neck cancer, and in 67 % of saliva samples from OSCC patients, according to direct sequencing. In 50–70% of epithelial tumours, the p53 gene, which is found on chromosome 17p13.1, is mutated. The loss of heterozygosity of the p53 allele has been found in 22% of pre-cancer cases and 20% of oral cancer cases. Oral cancer has been reported to affect other genes linked to p53 and the cell cycle, such as p16, p27, p63, and p73, to variable degrees. Boyle et al discovered tumor-specific p53 mutations in 71% of saliva samples from patients with head and neck cancer using plaque hybridization[6].

In 23–67% of primary OSCCs, the cyclin-dependent kinase inhibitor 2A, which is implicated in the retinoblastoma pathway of the cell cycle, seems to be methylated. The CDH1 gene is involved in cell adhesion, increases metastasis when mutant, and is methylated in the promoter in up to 85% of tumours. Rosas et al. discovered promoter hypermethylation in 65% of matched saliva samples from OSCC patients and found aberrant methylation of at least one of the genes p16, O6-methylguanineDNA methyltransferase, or death-associated protein kinase in OSCC patients. c-MYCIN-MYC has been shown to be amplified and overexpressed in 20–40% of oral malignancies[29]. Das et al. have reported amplification of 11q13, which contains 1NT2, HST1, and cyclin D oncogenes, in 30–50% of patients with oral cancer. The specificity and positive predictive value were higher for saliva than for serum[30].

Many investigations have found a substantial rise in salivary levels of Cyfra 21–1, tissue polypeptide-specific antigen, and cancer antigen 125 in patients with OSCC, with a sensitivity of 71%, specificity of 75%, negative value of 71%, and positive predictive value of 75%. Carcinoembryonic antigen and cancer antigen19-9, on the other hand, are not found with statistical significance[6].

## Transcriptomic mRNA

Salivary mRNA is thought to be stored in apoptotic bodies or actively released in exosomes or microvesicles. Researchers examined the clinical accuracy of salivary vs blood RNA biomarkers for oral cancer diagnosis and discovered four RNA biomarkers with a sensitivity and specificity of 91% and 71%, respectively, as well as a collective receiver operator characteristic value of 0.95. Seven mRNA molecules were discovered to occur substantially more often in OSCC patients than in healthy controls, according to research by Speight and Morgan[6]. These included the following:

1. IL8.
2. IL-1β, which takes part in signal transduction, proliferation, inflammation, and apoptosis.
3. Dual specificity phosphatase 1, which has a role in protein modification, signal transduction, and oxidative stress.
4. H3 histone, family 3A, which has DNA binding activity.
5. Ornithine decarboxylase antizyme 1, which plays a part in polyamine biosynthesis (vi) S100 calcium binding protein P, which has a role in protein binding and calcium ion binding.
6. Spermidine/spermine N1-acetyltransferase, which takes part in enzyme and transferase activity[6].

## Proteomic Substances

Carbonylation is a marker for oxidative damage to proteins, and there is a 246% rise in salivary carbonyls in OSCC patients, indicating that their epithelial cells are being attacked by free radicals. For carbonyls, the sensitivity and specificity are % and 80%, respectively. The MMP-9 polymorphism has been found to be significantly linked to an elevated risk of OSCC[6].

In OSCC patients, Shpitzer et al. found a 39 % rise in MMP-9 with a sensitivity of 100 % and specificity of 79%[31]. The concentrations of IL-6 and IL-8 in the saliva of OSCC patients were shown to be considerably higher by St John et al. and Rajkumar et al[32]. Another study reported that patients with OSCC have significantly higher concentrations of IL-8 in saliva than patients with dysplastic oral lesions and normal controls, suggesting its diagnostic value as a marker of malignant transformation of oral premalignant lesions. Arellano-Garcia et al. used Luminex xMAP (Austin, TX, USA) technology to show that both IL-8 and IL-1β are expressed significantly more strongly in OSCC patients[6].

## Light-Based Detection Systems

1. Chemiluminescence (ViziLite Plus; Microlux/DL,Orascoptic-DK)
2. Tissue fluorescence imaging (VELscope)

## Chemiluminescence

Clinical examination of the oral mucosa using chemiluminescent blue/white light has recently been recommended as a way to enhance the detection of mucosal abnormalities when compared to using standard incandescent light[1].

## Vizilite Plus

This relevant technology, the ViziLite system (Zila Pharmaceuticals, Phoenix, AZ), incorporates a one-minute oral rinse with a 1 % acetic acid solution, followed by a diffuse chemiluminescent blue/white light inspection of the oral mucosa (wavelength of 490–510 nm)[1]. The theory behind this approach is that acetic vinegar dissolves the glycoprotein barrier and mildly desiccates the oral mucosa, causing aberrant mucosal cells to absorb and reflect blue/white light differently than normal cells. The normal epithelium takes on a blue tint, but the acetowhite lesions are bright white[1].

Lumenoscopy also includes a tolonium chloride solution (toluidine blue), which is used to help designate the acetowhite lesion for biopsy after the light source is turned off. As an adjuvant to the traditional head, neck, and soft tissue examination, lumenoscopy with toluidine blue in oral lesion detection and labelling system is utilised[1,33].


**Fig. 8: (a) Oral screening with vizilite plus (b) MicroLux DL (c) Orascoptic DK (image courtesy to Aggarwal A et al, Oral lumenoscopy an adjuvant in early screening of oral cancer, Journal of Indian Academy of Oral Medicine and Radiology; 2011, 23(2):124-127).**

The VizLite system was recently updated to accommodate the usage of toluidine blue, as well as the introduction of the MicroLux DL, a new chemiluminescence device. It's worth noting that no research has shown that chemiluminescence can assist distinguish between dysplasia/carcinoma and benign tumours. As a result, the majority of research has focused on how chemiluminescence improves subjective clinical evaluation of intra-oral lesions, such as brightness, sharpness, and texture, in comparison to normal clinical examination[1]. Some authors claimed that this technique improved the detection of intra-oral abnormalities, while others claimed that the overall detection rate was not significantly improved, and that the chemiluminescent light produced reflections that made visualization even more difficult than with incandescent light. Methodological issues, such as a lack of histological diagnosis or specific aims, restrict the majority of investigations. Some studies claim that chemiluminescence can assist detect occult lesions that aren't visible under incandescent light, however there isn't much data to back this up[1].

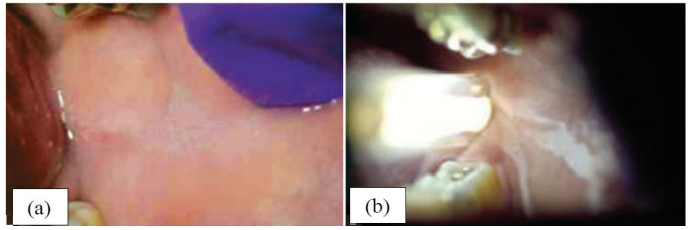



**Fig. 9: Visualisation of lesion in buccal mucosa (a) without Vizilite plus (b) with Vizilite plus (image courtesy to Aggarwal A et al, Oral lumenoscopy an adjuvant in early screening of oral cancer, Journal of Indian Academy of Oral Medicine and Radiology; 2011, 23(2):124-127).**

## Tissue Fluorescence Imaging

Tissue autofluorescence has been used in the screening and diagnosis of precancers and early cancer of the lung, uterine cervix, skin and, more recently, of the oral cavity[34].

Tissue autofluorescence has been used to explore the molecular features of cells and tissue because it has the ability to offer information regarding biochemical, functional, and structural changes of fluorescent bio-molecular complexes in vivo. Fluorescence has been widely investigated as a tool for tissue diagnosis and detection of malignant transformation due to the fact that pathological transformation, therapeutic treatments, and developmental alterations all generate biological changes in afflicted tissues[6].

When cells interact with a certain wavelength of light, they get stimulated and re-emit light of varying wavelength or colour, which is known as autofluorescence. Fluorophores, which are found naturally in most human tissues, cause autofluorescence of tissues. Collagen, tryptophan, elastin, keratin haemoglobin, and NADH are examples of naturally occurring fluorophores. The concentration of these fluorophores changes as a result of potentially malignant diseases and carcinogenic situations. They also induce changes in the tissues' inherent light scattering and absorption capabilities. Changes in the mucosa's spectral quality can be identified and utilised to diagnose possibly malignant diseases and cancerous situations[7].

The idea behind tissue autoflorescence is that changes in the epithelium's structure (e.g., hyperkeratosis, hyperchromatin, and increased cellular/nuclear pleomorphism) and metabolism (e.g., concentration of flavin adenine dinucleotide[FAD] and nicotinamide adenine dinucleotide[NADH]), as well as changes in the subepithelial layers. These epithelial and stromal alterations might affect the distribution of tissue fluorophores and, as a result, how they release fluorescence after being stimulated with strong blue excitation light (400–460 nm), a process known as autoflorescence[1,2]. Finally, a human observer may see the autoflorescence signal directly. When observed through the instrument handpiece, normal oral mucosa emits a pale green autofluorescence, but aberrant tissue emits less autofluorescence and appears darker in comparison to the surrounding healthy tissue. LED Medical Diagnostics Inc., in collaboration with the British Columbia Cancer Agency, developed autoflorescence technology for oral mucosa examination, which is marketed as the VELscope system[1,2].

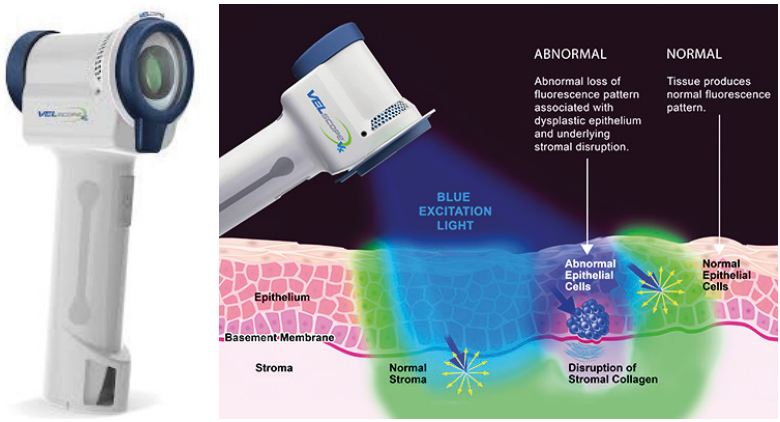



**Fig. 10: Tissue fluorescence using VELscope (courtesy to https://velscope.com/velscope/velscope-technology/tissue-fluorescence/).**

Several studies have investigated the effectiveness of the VELscope system as an adjunct to visual examination for:

1. Improving the distinction between normal and abnormal tissues (both benign and malignant changes).
2. Differentiating between benign and dysplatic/malignant changes.
3. And identifying dysplastic/malignant lesions (or lesion's margins) that are not visible to the naked eye under white light.[1]

Although more studies on diverse patient populations is needed, autofluorescence imaging of the oral mucosa has been shown to possibly enhance lesions contrast and hence increase the capacity to discern between mucosal lesions and healthy mucosa. The capacity of autofluorescence to distinguish between distinct lesion types has been studied in a few studies, and the method appears to have a high sensitivity but a low specificity[1]. The VEL scope technology, on the other hand, appears to be highly promising because of its capacity and efficacy in recognising lesions and lesions margins that are not visible under white light. VELscope revealed significant sensitivity and specificity in detecting regions of dysplasia and malignancies that extended beyond clinically visible tumours, using histology as the gold standard. Assessing lesion margins in patients with possibly malignant oral problems as a direct clinical application improves surgical therapy[1].

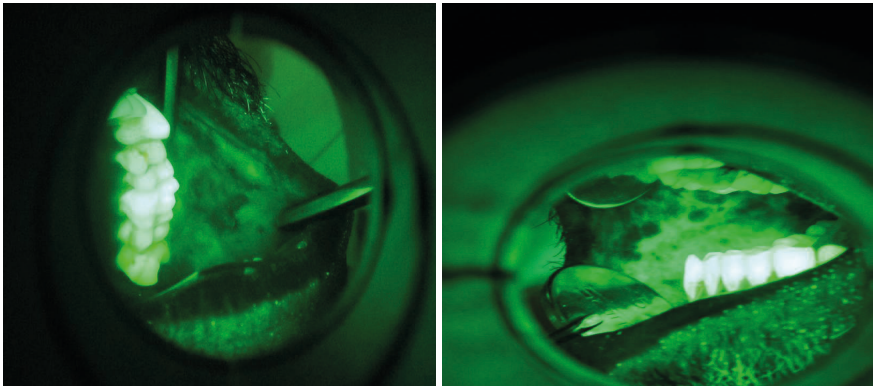


**Fig. 11: Velscope images of variations visualized on (a) left buccal mucosa and (b) right buccal mucosa (image courtesy: clinical photograph collected from Department of Oral Medicine and Radiology, JSS Dental College and Hospital, JSSAHER, Mysuru.**

# Advanced Diagnostic Aids

## Optical Biopsy

### *Tissue Fluorescence Spectroscopy*

Autofluorescence spectroscopy is a non-invasive approach for detecting structural and chemical changes in the oral mucosa that is quickly gaining popularity. Oral cancer and potentially malignant illnesses are invariably linked to structural and biochemical changes in the mucosa. Molecular changes occur even before clinical indications of these lesions. Modifications in the mucosa's optical properties may provide insight into these early molecular level changes. Autofluorescence spectroscopy, as opposed to autofluorescence imaging, may detect even slight mucosal changes[7].

The autofluorescence spectroscopy system comprises of a tiny optical fibre that generates various excitation wavelengths and a spectrograph that collects and records the spectra of reflected fluorescence from the tissue on a computer and analyses it using special software. This method has the obvious benefit of removing the need for subjective assessment of tissue fluorescence variations. Overall, autofluorescence spectroscopy appears to be quite good at differentiating lesions from healthy oral mucosa, with excellent sensitivity and specificity, especially when comparing malignant tumours to healthy mucosa[1,2]. However, it has been noted that the technique's capacity to differentiate and classify distinct forms of lesion is limited. Furthermore, while the autofluorescence approach is quite effective in discriminating normal mucosa from various diseases, scanning extensive regions of oral mucosa is not practicable due to the tiny size of the optical fibre. As a result, spectroscopy can only be used to evaluate a well-defined tiny mucosal lesion that has previously been recognised by visual inspection. It also can't tell the difference between benign and malignant tumours[1,2].

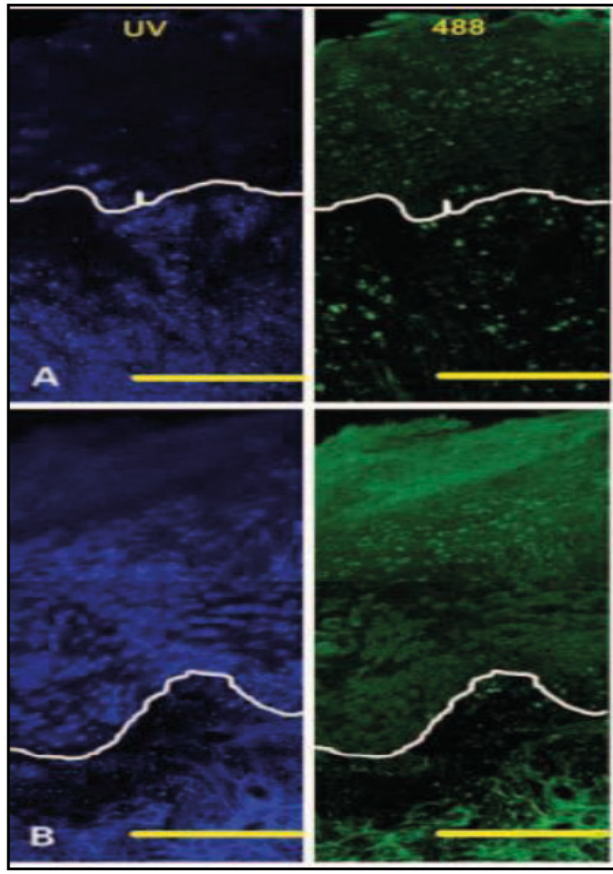



**Fig. 12: (a) Fluorescence patterns of inflammation and (b) Mild dysplasia in the tongue. (image courtesy to Williams M et al Understanding the Biological Basis of Autofluorescence Imaging for Oral Cancer Detection: High-Resolution Fluorescence Microscopy in Viable Tissue Ina Pavlova, Clin Cancer Res 2008;14(8) April 15, 2008).**

## Enhanced Dye Fluorescence

Exogenously administering fluorescent medications such as 5-aminolevulinic acid (5-ALA), which causes protoporphyrin IX (PPIX), a precursor to physiologically necessary prosthetic groups such as heme, cytochrome c, and chlorophylls, can marginally boost fluorescence. Recent improvements include the ability to quantitatively eliminate the unwanted effects of scattering and absorption to obtain genuine spectra of single fluorophores (chemical substances that may re-emit

light upon light stimulation). Furthermore, fluorescent indicators ("smart probes") will soon be able to specifically target tumor-specific enzymes, improving both sensitivity and specificity[6].

## Raman Spectroscopy

The Raman effect was first discovered by Professor Raman of Calcutta University, for which he was awarded the Nobel prize in 1930[35]. This effect is based on the interaction of light with matter; when photons are aimed at target materials, the majority of them pass through unaltered. Some photons, however, collide with molecules in the substance. The majority of these photons interact with the substance's molecules, causing them to enter a partial quantum state and emit photons with the same frequency as the incident photon[36]. Elastic scattering is the name for this process. A smaller number of these photons are subjected to a process known as Raman or inelastic scattering, in which photons are released from the material or scattered at a wavelength different from the input photon; Raman spectroscopy records this wavelength shift. This approach can provide biochemical and morphological information in a short amount of time, which can be utilised for diagnosis[6].

The advantage of Raman spectroscopy is the use of optical fibres for guiding the laser light to the desired site and also it helps in the in vivo measurements by collecting these Raman photons. It can, therefore, be used to record the in vivo spectra of tumor cells and normal cells of the same subject to provide a diagnosis in the least possible time[3].

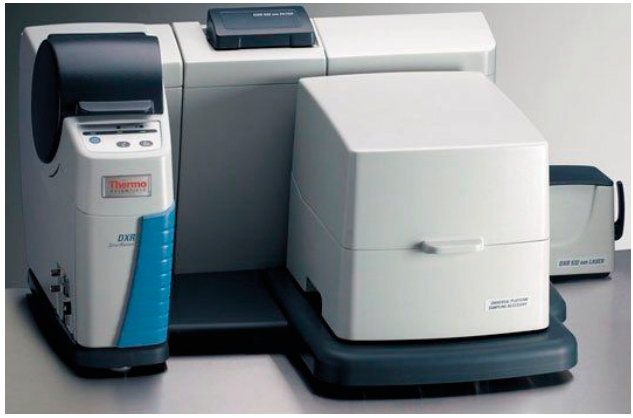



**Fig. 13: Raman spectrometer (image courtesy to http://img.directindustry.com)**

A laser-based spectroscopic approach for viewing vibrational, rotational, and other low-frequency modes in a system has been created, allowing chemical and molecular structure to be characterised in a sample. Laser light interacts with molecular vibrations, phonons, or other excitations in the system, shifting the energy of the laser photons up or down in the process. These energy transfers reveal information about the system's vibrational modes. This method produces a vibrational spectroscopic image of tissue composition, allowing for real-time histology[37,38,39].

The signals produced by the Raman effect are intrinsically weak, and Raman bands frequently overlap due to diverse biological ingredients, making it difficult to precisely identify specific components. Biomedical samples provide a strong fluorescence background that can entirely mask the real Raman signals[6].

According to several research, Raman spectroscopy has the potential to increase the effectiveness of oral cancer screening techniques and to determine the tumor-free resection margin during surgery. Several studies have shown that laser-induced fluorescence (LIF) may be used to differentiate between normal and cancerous tissues. However, extracting bio-chemical information from wide fluorescence bands is difficult, whereas Raman spectra readily provide such information, making them more ideal for diagnostic applications. With present day developments in technology, Raman spectroscopy has sensitivity similar to fluorescence and may thus be utilised frequently for diagnostic purposes. For optical pathology of oral tissue, Raman spectroscopy can be utilised to identify malignancy. The approach is quick, may be used in real time, and can be paired with data processing techniques to get highly objective results[6].

## Elastic Scattering Spectroscopy

Instead of relying on subjective picture interpretation, elastic scattering spectroscopy (ESS) uses objective statistical and analytical approaches to make diagnoses. It delivers white light reflectance-based optical geometrical information. Photons hit tissue and scatter back without changing wavelength in elastic scattering spectroscopy. The nature of the probed tissue, especially the relative concentration of scatterers (e.g., nuclei, mitochondria, connective tissue), influences the relative intensity of this back scattering (e.g., haemoglobin). All of the features of the cellular components, known as "scattering centres," are carried by a scattering event. Pathological scattering centres can be caused by disorganised epithelial orientation and architecture, changes in epithelial surface thickness and texture, cell crowding, increased distance from the subepithelial collagen layer, enlargement and hyperchromicity of the cell nucleus, increased concentration of metabolic organelles, and the presence of abnormal protein packages or particles[38,39].

Without photographing the microscopic structure, the ESS approach detects micromorphology changes at the level of subcellular architectural modifications, such as nuclear grade, nuclear to cytoplasmic ratio, mitochondrial size and density. Because ESS identifies alterations at the subcellular level, it provides information that traditional histology may not[6].

As a result, ESS gives an optical signature of a tumour that is very dependent on its form. The ESS system employs a pulsed xenon arc lamp to span a wavelength range of 300–900 nm (light produced by cellular and subcellular organelles varies from 330–850 nm). Two fibreoptic probes are used in the system, one to transmit light into the tissue and the other to gather scattered light. The collecting probe's tip is positioned directly on the lesion, and a background measurement is obtained before the light is turned on. The pulsed light is then used to obtain an ESS measurement in less than 100 milliseconds, following which the background spectrum is removed from the ESS spectrum. The entire measurement processing display takes less than 1 second[37,38,40]. Thus ESS provides a point measurement that uses appropriate optical geometry and is sensitive to the size and packing of dense subcellular components (such as the nucleus, nucleolus, and mitochondria) as well as absorption by haemoglobin[6].

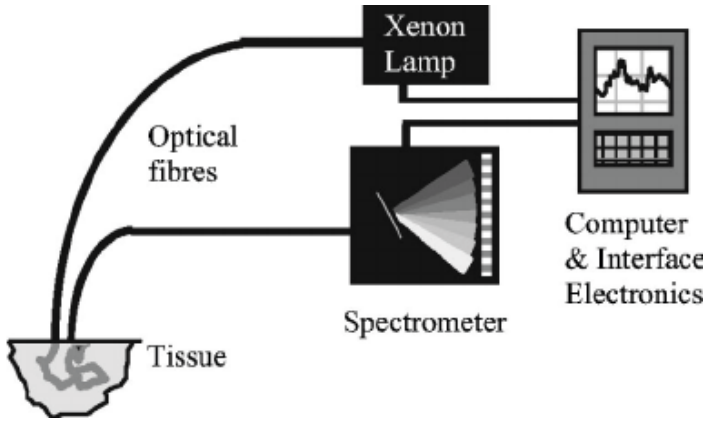



**Fig. 14: Schematic diagram of elastic scattering spectroscopy (ESS) system. (image courtesy to https://www.researchgate.net/figure/Schematic-diagram-of-elastic scattering-spectroscopy-ESS-system_fig2_8158492)**

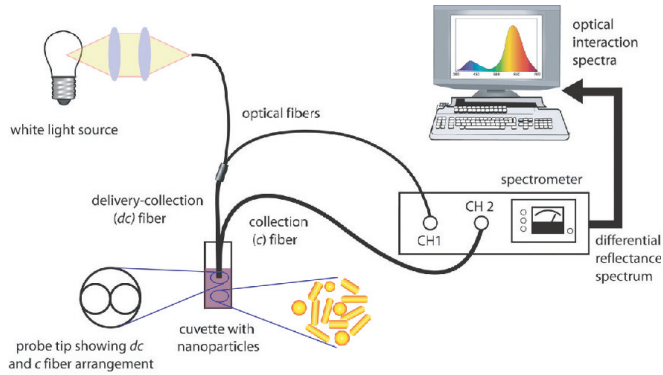
The study of malignancy and dysplasia of the oral cavity using ESS is in development. Ex vivo work by Jerjes et al[39] used the technique to study formalin-fixed specimens of cervical lymph nodes and bony margins taken from patients

with oral squamous cell carcinoma. Using linear discriminant analysis they showed sensitivity and specificity of 98% and 68%, respectively for the lymph nodes and 87% and 80% for the bone margins. The same group suggested that ESS has the potential to perform a full optical mapping of the suspicious area, thus eliminating the need for pathology[5].

Muller et al. used ESS to look at normal versus abnormal tissue and dysplastic versus cancerous in the oral cavity. When comparing spectroscopy to histopathology, the accuracy for normal tissues was 91.6% (22/24) compared to 97% (33/34) for abnormal tissues. These figures fell when examining dysplasia, 64.3% (9/14) and carcinoma 50% (5/10). However, when using tri-modal spectroscopy, Muller et al showed a sensitivity and specificity of 96% when comparing cancerous/dysplastic tissues from normal tissues and obtained values of 64% and 90%, when comparing dysplastic with cancerous tissues, respectively[5].

## Differential path-length spectroscopy

Differential path-length spectroscopy is a fiberoptic point measuring technique that samples tissue in a predetermined sampling volume and counts scattered photons that have travelled predetermined route lengths. The spectrum is statistically examined, and the results are converted into a set of parameters relating to microvasculature and intracellular morphology[5]. Differential path-length spectroscopy is considered to be a form of ESS that has fixed photon path length, fixed photon visitation depth, and absolute measurement of absorbers. This technology was developed at the Erasmus Medical Centre, Rotterdam, the Netherlands[6].




ferential pathlength spectroscopy experimental setup.

**Fig. 15: Differential pathlength spectroscopy experimental setup (image courtesy to https://www.semanticscholar.org/paper/Differential-pathlength-spectroscopy-for-the-of-of-Ungureanu-Amelink/aadd009acf4087184e8260a864caac434bbb45d4)**

A diffuse reflection spectrometer with a tungsten-halogen lamp as a white light source is used in the system. For lighting and collection, the first spectrometer employs a bifurcated fibre. Diffusely reflected light is sent to a second spectrometer through a second cable. Each spectrometer's recorded spectra have somewhat varied wavelength scales. The light that is superficially dispersed is selected by subtracting the two measurements[41].

This spectrum is analysed mathematically and translated into a set of parameters that are related to the microvasculature and intracellular morphology. The signals give information about cell biochemistry, intracellular morphology and microvascular properties such as oxygen saturation and average vessel diameter. The reported sensitivity is 69% and specificity is 85%[41].

Amelink et al. used differential path-length spectroscopy to study 76 spectra (45 nondysplastic and 31 dysplastic) collected from 27 leukoplakias. Based on a combination of the three variables of blood oxygenation, vessel diameter, and blood volume fraction, nondysplastic and dysplastic leukoplakias can be discriminated with a sensitivity and specificity of 91% and 80%, respectively[41].

## Nuclear Magnetic Resonance Spectroscopy

The magnetic characteristics of particular atomic nuclei are used in nuclear magnetic resonance spectroscopy (NMR) to identify the physical and chemical properties of atoms or the molecules in which they are included. It is based on the nuclear magnetic resonance phenomenon and may offer extensive information on a molecule's structure, dynamics, reaction state, and chemical environment[6].

The magnetic properties of atomic nuclei are used in nuclear magnetic resonance (NMR) spectroscopy, which is a quantitative approach. NMR active nuclei (e.g., 1 H and 13 C) absorb electromagnetic radiation and transition from a low-energy spin stage to a high-energy spin stage when the sample is put in an external magnetic field. The nuclei release electromagnetic radiation and return to a low-energy state when subjected to radiofrequency pulses[42].

The nuclei are considered to be in resonance with the magnetic field outside of the nucleus. NMR spectroscopy is a powerful method for identifying tiny molecules in bodily fluids such as saliva because the resonance frequencies and chemical shifts are unique or highly specific to particular components. NMR spectroscopy also enables for quantitative investigation of salivary metabolites

since the area beneath a signal peak is related to the concentration of a specific molecule. The discovery of novel salivary biomarkers would allow us to detect HNSCC in its early stages, which would be extremely beneficial and might aid in the selection of the best treatment options[6,42].


**Fig 16: Nuclear magnetic resonance spectroscopy (Image courtesy to https://en.wikipedia.org/wiki/Nuclear_magnetic_resonance_spectroscopy)**

The greater the magnet, the more sensitive the gadget. This technique permits three-dimensional examination of atoms in molecules. It is possible to see how proteins interact with DNA using NMR. When comparing OSCC to normal tissues, NMR was employed to determine metabolic markers. The choline/creatine ratio in OSCC is much greater than in normal tissue, according to clinical trials. Taurine, choline, glutamic acid, lactic acid, and lipids were shown to be greater in SCC than in normal tissue in an NMR examination of ex vivo cancer tissue. In addition, OSCC has been linked to overexpression of glucose transporters, particularly glucose transporter 1, which is linked to enhanced glycolytic metabolism. Other researchers have found that the expression of advanced glycated end-products and higher numbers of their receptors correlates significantly with the invasiveness of OSCC in individuals with primary gingival cancer[6,42].

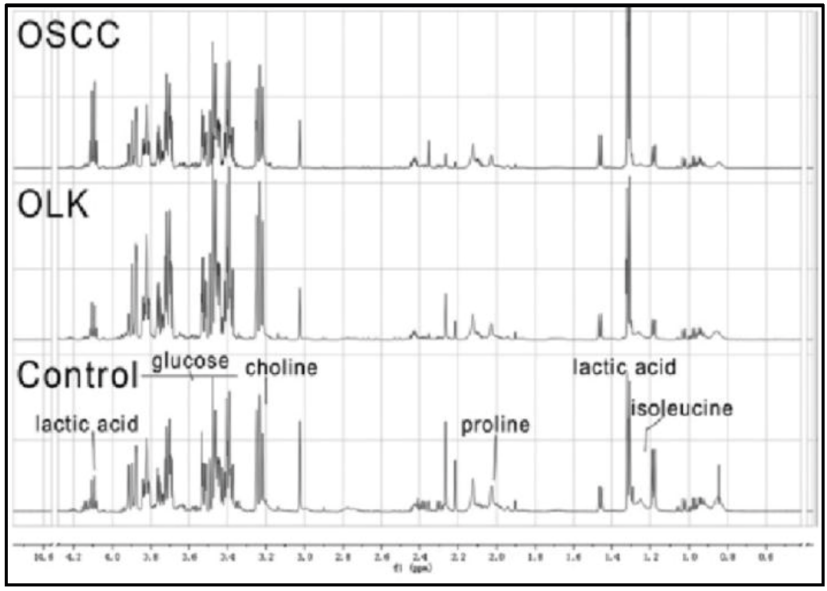



**Fig. 17: Representative 1 H nuclear magnetic resonance spectra of rat plasma at various stages of oral carcinogenesis. OSCC, oral squamous cell carcinoma; OLK, oral leukoplakia.**

(Image courtesy to Kong et al, Analysis of plasma metabolic biomarkers in the development of 4nitroquinoline1oxideinduced oral carcinogenesis in rats, Oncology letters 9: 283-289, 2015).

## Confocal Reflectance Microscopy (CRM)

CRM (confocal reflectance microscopy) is a non-invasive optical approach of visualising tissue. CRM provides for high-resolution imaging of intact tissue by imaging light reflected from a specified depth inside the tissue while rejecting light from layers above and below that plane. CRM differs from a traditional microscope in that it illuminates a tiny location within tissue using a point source of light, usually a laser. Back-scattered or reflected light from the tissue is caught through an aperture that is the same size as the lighted area in front of the detector. Only the plane in focus is photographed because the aperture spatially filters light returning from out-of-focus planes within the tissue[43].

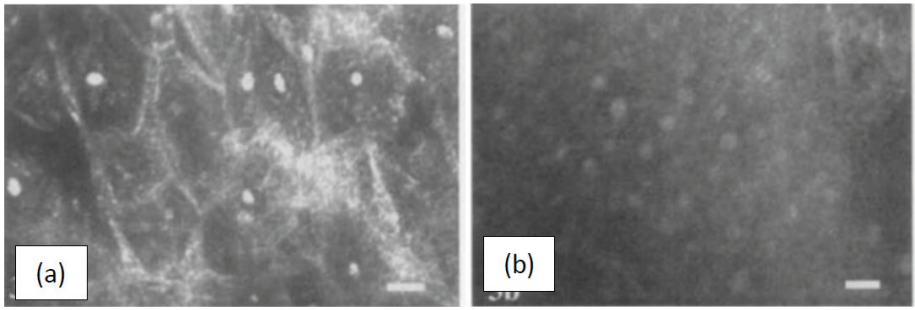



**Fig. 18: Confocal reflectance images of human lip mucosa in vivo.(a) the superficial epithelial cell layer at a depth of 50 µm; (b) deeper epithelial cell layer at a depth of 98 µm (image courtesy to White et al, Noninvasive Imaging of Human Oral Mucosa in Vivo by Confocal Reflectance Microscopy,Laryngoscope 109: October 1999).**

In vivo fibre optic confocal reflectance microscopy has significant clinical potential to provide a sensitive and specific method for non-invasive detection of precancer and cancer in the oral cavity[43].

## Optical Coherence Tomography

Optical coherence tomography (OCT) is a non-invasive imaging technique that allows for cross-sectional imaging of biological tissue. Because both methods use back scattered signals reflected from different levels inside the tissue to build structural pictures, OCT is frequently likened to ultrasound imaging. Optical coherence tomography (OPT) is a type of imaging that employs light instead of sound. Unlike traditional medical imaging modalities, OCT produces high-resolution (micrometre scale) pictures in real time. Flexible fibreoptic OCT probes can be used to capture these pictures noninvasively in vivo[44].

OCT's great spatial resolution allows for noninvasive in vivo "optical biopsy" and gives rapid and precise diagnostic information. The recent invention of a Fourier domain mode lock swept source-based OCT system has aided in achieving high speed (>100 kHz A-scan rate) and good spatial resolution (>4 m) at the same time. Furthermore, the invention of a number of small scanning probes capable of producing high-speed three-dimensional OCT images has been described. To see if OCT might be used to detect malignant tissue, Jerjes et al. matched OCT data to histological diagnoses of suspected mouth lesions. With swept-source frequency domain OCT, 34 oral lesions from 27 individuals were evaluated. A total of four factors were examined (changes in keratin, epithelial, and subepithelial layers, and identification of the basement membrane)[6].

The viability of employing OCT to detect architectural alterations in malignant tissues was validated by these researchers. Olivo et al. found a high level of agreement between OCT-based and histological diagnoses, with sensitivity and specificity ranging from 93–97%, respectively. Volgger et al. looked at how well OCT could distinguish between premalignant and early malignant lesions in the upper aerodigestive tract. Noninvasive and invasive lesions were discriminated with a sensitivity of 88.9% and specificity of 89.0%. In a hamster cheek pouch model, they demonstrated the potential of employing quantitative image analysis algorithms to extract morphological information from OCT pictures in order to make automated diagnoses of oral cancers. The inclusion of OCT with specialised equipment, such as hand-held and catheter-based probes, will increase the technology's use in clinical practise[6].

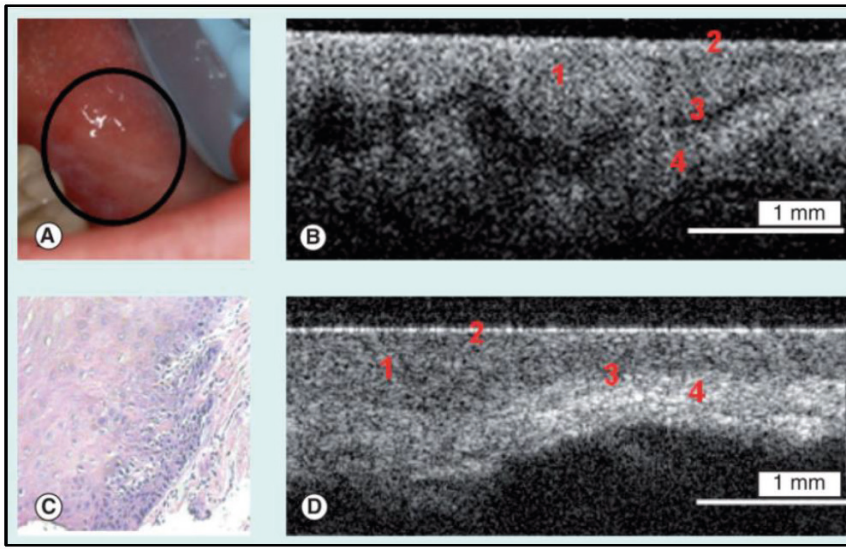



**Fig. 19: Dysplastic and normal buccal mucosa (A) Photograph, (B) in vivo optical coherence tomography image and (C) hematoxylin and eosin (10×) of dysplastic buccal mucosa. (D) In vivo optical coherence tomography image of normal buccal mucosa. 1: Stratified squamous epithelium; 2: Keratinized epithelial surface layer; 3: Basement membrane; 4: Submucosa.**

(Image courtesy DeCoro M et al, Potential of optical coherence tomography for early diagnosis of oral malignancies, Expert Rev Anticancer Ther. 2010 March; 10(3): 321–329).

Early clinical experiments employing in vivo OCT imaging have shown the promise for OCT-based oral diagnosis and the development of a good diagnostic algorithm for mapping oral lesions based on OCT scan data. As a result, these findings support the idea that OCT will be a valuable tool for early identification and diagnosis of oral lesions, as well as frequent monitoring of suspicious oral lesions and quick, low-cost screening of high-risk groups[44].

In a recent independent, blinded study, the clinical diagnostic capability of in vivo OCT for oral dysplasia and malignancy was investigated in 50 patients with oral leukoplakia or erythroplakia lesions. The OCT image of a dysplastic lesion parallels histopathological status, showing epithelial thickening, loss of stratification in lower epithelial strata, epithelial downgrowth and loss of epithelial stratification as compared with healthy oral mucosa. Statistical analysis of the data confirmed the capability of in vivo OCT for detecting and diagnosing oral premalignancy and malignancy in human subjects[6,44].

For detecting carcinoma in situ or SCC versus non-cancer, sensitivity was 93% and specificity was 93%; for detecting SCC versus all other pathologies, sensitivity was 93% and specificity was 97%[6].

## Angle-Resolved Low Coherence Interferometry (A/LCI)

Angle-resolved low coherence interferometry (A/LCI) is a new biomedical imaging technique that uses the properties of scattered light to determine the average size of various cell structures, including cell nuclei, and directly measures diagnostically relevant subcellular features of epithelial tissues up to 500 m below the surface. Unlike OCT, which is a subjective approach that needs image interpretation, A/LCI analyses tissue objectively and provides physicians with immediate proof of precancerous illness. Wax et al. matched the average diameter and texture of cell nuclei in rat esophageal epithelial tissue to grading criteria used in a prior a/LCI research to prospectively evaluate neoplastic development[6].

Using histology as the gold standard, the combined investigations revealed 91% sensitivity and 97% specificity for identifying dysplasia. According to Chalut et al., the a/LCI approach has a sensitivity of 78% and a specificity of 91% in distinguishing normal from sick tissue. Terry et al. looked at samples from 27 individuals who had partial colonic resection surgery to see if a/LCI could detect dysplasia. They found that a/LCI had a sensitivity of 92.9% (13/14), a specificity of 83.6%, and an overall accuracy of 85.2% in distinguishing dysplastic from healthy tissues. According to Zhu et al., a/LCI was able to identify dysplasia with 100% sensitivity and 84% specificity in 46 individuals with Barrett oesophagus[6].

Wax et al. devised a new spectroscopic approach based on low-coherence interferometry to detect the angular distribution of scattered light for detecting illness at the cellular level. Low-coherence interferometry's capacity to separate scattering from underlying tissue layers is combined with light scattering spectroscopy's ability to extract structural information on subwavelength scales in A/LCI. The device has potential as a clinical tool for detecting dysplastic or precancerous tissue in real time[6].

## Thernostics

The word "Thernostics" is a new concept that refers to the use of diagnostic indicators to guide treatment decisions with the goal of personalising patient care. These diagnostic indicators are frequently molecular in origin and are sometimes referred to as predictive markers since they can predict clinical outcome (prognosis) or therapy response. Thousands of researches have found a link between the molecular level of certain indicators in tumour tissue and bodily fluids and the advancement of cancer. Biomarkers, which are defined as genomic, proteomic, and metabolomic profiles of body specimens, play a significant role in determining the existence or absence of a certain malignancy, as well as its spread and recurrence[45,46].

Nanoscience and nanotechnology, as sciences concerned with the study and use of extremely small things, provide novel and ground-breaking approaches to cancer therapy and diagnostics. In recent years, there has been a lot of interest in site-specific chemoprevention/therapy employing nanoparticles for local drug delivery. Nano-therapy has evolved to cancer treatment using new ways in order to overcome and reduce the limitations of present cancer treatment and diagnostics. It has the capacity to identify even a single cancer cell in vivo and deliver medications directly to malignant cells. Nanotechnology has also enhanced the identification of cancer biomarkers, making them more quick and sensitive. Following a definitive diagnosis of oral cancer, OSCC is usually treated with a multidisciplinary strategy that includes surgery, chemotherapy, and radiation. Conventional chemotherapeutic drugs, on the other hand, may be inefficient and have a variety of adverse effects. To address these disadvantages and enhance in situ medication administration, many nanoparticles have been used as technical drivers of innovation[47].

## Nano-Based OSCC Biomarker Detection

Biomarkers with low quantities in tissue samples or body fluids can be detected more sensitively using nanotechnology. The saliva peptide finger print analysis

has been proposed as a new approach for salivary proteomics analysis as well as possible biomarkers prediction for cancer diagnosis. Magnetic nano-beads have been reported to be used to enhance low-molecular-mass peptides selectively[45]. This technique has offered a new non-invasive plan for screening oral cancer biomarkers, with features such as low cost, easy purification, and high sensitivity, allowing it to capture more proteomes than previous methods, particularly in the low-molecular-mass range. This method allows for the identification of detailed 'fingerprints' of protein patterns in biological fluids, which may be utilised to discover disease biomarkers. The adaptation, downsizing, integration, and automation of analytical laboratory operations into a single device or electronic chip is referred to as single biomarker detection technique. The method is based on an enzyme-linked immunosorbent test (ELISA)[45].

TNF- was detected by Omar et al utilising a gold protein chip and total internal reflection fluorescence microscopy (TIRFM) as a nano-based single biomarker detection approach for oral cancer diagnostics[48].

According to studies, this approach may detect oral cancer at the attomolar (aM) concentration level (1018), resulting in great sensitivity[45]. Weigum et al. describe a nano-bio-chip (NBC) sensor approach for investigation of oral cancer biomarkers in exfoliative cytology specimens, addressing both biochemical and morphologic alterations associated with early oral carcinogenesis in yet another research on oral cancer[49]. Using established biomarker and cytomorphometry assays for the epidermal growth factor receptor, specimens were enriched, immunolabeled, and imaged in the NBC sensor (EGFR). According to studies, this approach may identify oral cancer at the attomolar (aM) concentration level (1018), resulting in great sensitivity. The use of a nanobiosensor based on chip technology to examine the oral cancer biomarker EGFR with exfoliated cytology specimens of OPMD or OSCC patients has also been described. According to the findings, the amount of EGFR expression, as well as nuclear area, nuclear diameter, and nuclear-to-cytoplasmic ratio, was significantly altered in oral lesions with squamous cell carcinoma or dysplasia[49].

## OSCC Treatment Strategies Based On Nanomaterials

Conventional therapy techniques to treating OSCC are associated with a number of adverse effects that can be both temporary and permanent. Oral carcinogenesis

has been proven to respond well to novel treatment techniques. This group might include therapeutic compounds such as siRNAs and other active targeting ligands. Several natural compounds have also shown promise in the treatment of oral cancer by interfering with several cell signalling pathways such as free radical scavenging, inhibiting the production of DNA adducts, and regulating the characteristics of apoptosis-related genes. However, these chemicals' poor bioavailability and solubility have limited their clinical utility[45].

For the development of drug delivery systems, the time of drug engagement with oral tumour cells is a critical consideration. As a result, a sustained and targeted mucoadhesive drug delivery system into the oral cavity might be a viable option. Nanoparticle residence duration can lead to improved drug–mucus interaction and mucoadhesion. The studies also demonstrated that local distribution of nanoparticles for site-specific chemoprevention/therapy can increase targeting into the oral cancer site by reducing nonspecific drug absorption by cells[45].

Nanoparticle-based cancer treatment also has the advantage of inhibiting quick clearance by the reticuloendothelial system (RES) and improving plasma half-life, which leads to lower medication dose. Different types of nanoparticles have been tried for cancer treatment, according to scientific publications. Magnetic nanoparticles (MNPs), liposomes, polymeric nanoparticles, gold (Au) nanoparticles, and nano-diamonds are some of the most recent nanoparticles that have been studied for the treatment of drug-resistant cancer cells. Different nanotechnology technologies can also be utilised to target cancer biomarkers and cancer cells selectively. This option allows for sensitive and early cancer detection[45].

They aid early detection by concentrating and preserving a marker against deterioration. Using particular crosslinkers against cancer cells, such as antibodies or aptamers, can also help speed up the development of early detection tools. The various therapy techniques in oral oncogensis are summarised in Fig. 2. Furthermore, the creation of nanocarriers functionalized with various targeting agents (ligands, tumor-associated antigens, antibodies, and aptamers) has shown promising results in increasing the delivery of a specific target to cancer cells. This technique can consist of a single agent or a mix of agents that can be used in a variety of cancer immunotherapy or target drug/gene delivery routes[45].

## Biomarkers

Oral cancer detection has been the subject of several investigations. Currently, the most prevalent form of detection is cytological testing. Various light-based

oral cavity visual exams have been proposed for screening oral lesions in recent decades, however these procedures have significant drawbacks. Biochemical and molecular tests involving the oral mucosa, on the other hand, have the greatest promise for overcoming diagnostic constraints and would make good gold standards. It is critical and crucial to employ the substantial knowledge gained about oral cancer in the clinical setting[50,51].

The most accurate sign of malignant potential is histological evaluation of oral epithelial dysplasia (OED), however this is subjective and has a lot of inter- and intra-examiner variance in grading. As a result, there is a growing need to discover objective indicators that can differentiate between lesions that will advance and those that will not. Nuclear DNA content that is abnormal (aneuploidy) is a sign of chromosomal abnormalities and is linked to malignant and premalignant lesions. The occurrence of aneuploidy in tumours at an early stage shows that aberrant DNA content is linked to tumour growth and may be a precursor to cancer[52].

| Salivary biomarkers of oral cancer | |
|---|---|
| DNA | - Loss of heterogeneity: p53, 3p,9q, 13q and 17p<br>- Methylation: CDKN2, CDH1, MGMT, p16, DAPK<br>- Amplification: Cyclin D, cMYCIN-myc |
| RNA | - mRNA: Upregulated IL-8, IL-1β, H3 Histone, S100 calcium binding protein P<br>- miRNA: → Down regulated miRNA 200a and 125a; → Upregulated miRNA 31<br>- miRNA in malignancy → Down regulated miRNA 145; → Upregulated miRNA 184 |
| Protein | - IL-6 and IL-8<br>- Matrix metalloproteinase 2, 9, 11<br>- Immunoglobulin A and G<br>- Reactive nitrogen species, Nitric oxide, Oxidative Carbonylation<br>- Tissue polypeptide specific antigen<br>- Insulin growth factor<br>- Cytokeratin-19 fragment |

**Table 3: Different types of salivary biomarkers in oral cancer. E. Ahmadian et al,The potential of nanomaterials in theranostics of oral squamous cell carcinoma: Recent progress,Trends in Analytical Chemistry 116 (2019) 167-176.**

## Evaluation of Oral Cancer Biomarkers from Readily Available Samples:

Numerous biochemical alterations have been discovered with the start of oral cancer, according to research conducted over several decades. These biochemical indicators show a great deal of variation, which might be related to the patients' different lifestyles, genetic make-ups, and geographic origins. As a result, determining a standard set of biomarkers will be critical to diagnostic precision. Examining body fluids such as saliva and plasma can reveal important information about premalignant mouth lesions[51].

### Analyses Using Saliva

Saliva is an aqueous secretion of the oral cavity that contains live/dead cells, DNA, RNA, and metabolites or imprints from pre-existing neoplastic lesions. Many reports exist that imply a variety of alterations in saliva biochemical parameters that are suggestive of oral pre-cancer and hence effective in early identification. A truncated cystain SA-I found in saliva (with three amino acids removed from the N-terminus) has been proposed as a biomarker for oral cancers. In oral cancer patients, the expression levels of IL-8 and IL-1 beta in saliva were shown to be considerably higher than in healthy people. Furthermore, some salivary genes are methylated in afflicted people but not in healthy people, suggesting that they might be useful early indicators for oral cancer. Furthermore, variations in Cyfra 21-1 levels (a soluble fraction of cytokeratin 19 found in saliva) are closely linked to OSCC[51].

A number of additional saliva-based oral cancer-specific protein indicators have also been proposed. Combining these indicators might make it easier and more accurate to develop a salivary-based test for detecting oral pre-cancer. Microorganisms have been found to be implicated in different forms of cancer and are commonly present in salivary fluid; for example, the bacterium Helicobacter pylori is known to have a role in stomach cancer. More than 750 different bacteria live in the oral cavity, and the oral epithelium is continually exposed to a range of microorganisms. Similarly, a relationship has been shown between HPV and cervical cancer, and there is a growing body of research showing that OSCC is linked to HPV. In reality, the presence of Epstein-Barr virus (EBV) and human papillomavirus (HPV) infection may promote the spread of cancer. As a result, the oral mucosa includes pathogen-produced DNA, proteins, and metabolites that may help in the prediction of oral diseases[51].

Salivary diagnostics has long been an appealing diagnostic and screening alternative for clinical doctors and basic researchers as an excellent modality for early screening, diagnosis, prognosis evaluation, and therapy monitoring for oral and systemic disorders. Proteases are crucial molecules that cleave proteins at either the N-terminal or C-terminal domains, and they play a role in a variety of physiological and pathological processes. In humans, around 500–600 distinct proteases have been found. Depending on how they break down proteins, human proteases are classified as threonine, serine, cysteine, aspartic, or metalloproteases. Protease activation that is aberrant can cause pathogenic alterations in cells, tissues, and organs. Many proteases have been linked to the metastasis and translocation of human malignancies in previous studies. Inhibitors of these proteases can reduce cancer cell invasion and metastasis[53].

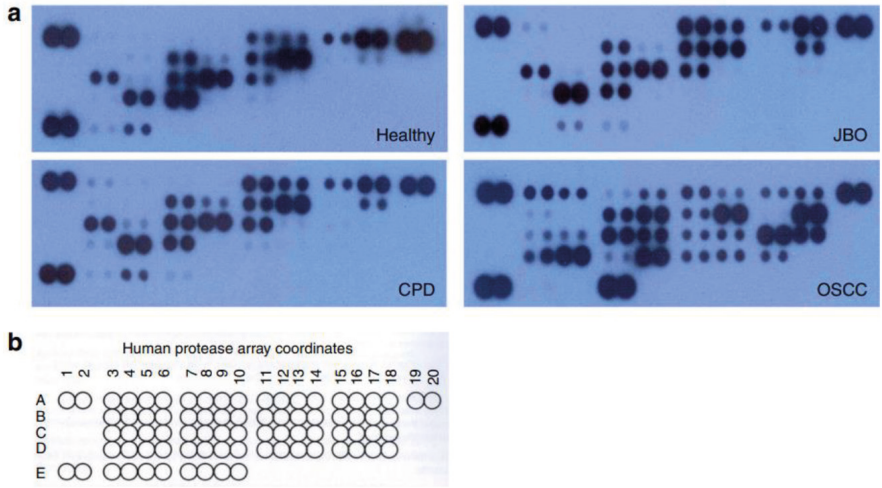



**Fig. 20: Analysis of salivary proteases by human protease array kits in healthy donors and patients with jaw bone ossification fibroma (JBO), oral squamous cell carcinoma (OSCC) and mild chronic periodontitis (CPD). Saliva proteases were detected using human protease array kits according to the manufacturer's instructions. (a) Protease profile analyses using protease array kits. (b) Each dot on the membrane represents a corresponding protease.**

## Analyses Using Serum

Blood connects all parts of the human body and transports various products and metabolites. The identification of circulating antigens might reveal information

about any disorders present, including OSCC. Many studies back up this theory, including one that looked at the oral cancer serum transcriptome. An increase in plasma microRNA miR-31 has been recommended as a marker for oral cancer, while circulatory VEGF has been reported as an indication. Overexpression of proteins such EGFR, cytokeratins, G protein-coupled receptor 87, Rab11 GTPase, PDZ domain containing proteins, and PEST-containing nuclear proteins has been found in the serum of patients with oral cancer. The p53 autoantibody was found in the serum of 25% of HNSCC patients[51].

Similarly, its presence in serum has been linked to lymph node metastases in other investigations. The prostaglandin E2 (PGE2) produced by cyclooxygenase (COX) is involved in the tobacco-induced carcinogenesis of OSCC. Furthermore, C16, C24, and C24:1-ceramide levels rise in OSCC, but C18-ceramide levels fall, showing their potential prognostic significance. More study is needed on the amounts of hormones and metabolites in patients' sera. Males are thought to be more vulnerable to OSCC than females. Although it is difficult to draw conclusions based on tobacco or alcohol abuse, various hormones and their receptors appear to play a role in the development of OSCC. Furthermore, women who have taken oestrogen in the past are less likely to develop oral leukoplakia, whereas the decrease in oestrogen that occurs after menopause makes women more susceptible to the condition. To validate these predictive parameters, more research is needed[51].

## Use of Molecular Biology in The Diagnosis Of Oral Cancer

The use of new, sophisticated procedures, similar to biochemical parameters, is improving information about the molecular properties of OSCC, which may enable the discovery of biomarkers and the design of improvised testing. Scalpel or brush biopsy can be used to obtain oral mucosa cells. To determine the state of oral lesions, the expression of important biomolecules such as DNA, RNA, and proteins can be measured[51].

## DNA-Based Alterations and Predictive Markers

The DNA that has been obtained from oral lesions may exhibit several changes, including amplification and LOH (loss of heterozygosity) (loss of heterozygosity). In addition, microsatellite instability, hot spot mutations, and epigenetic alterations (including methylation) of various oncogenes and tumour suppressor genes have been related with oral pre-cancer and cancer and are typically consistently used to screen pre-cancer. Different chromosomal segments are amplified and the copy

number increases during the amplification process. Oncogenes are mostly found in these chromosomal regions[51].

The most common loci that have been observed thus far in oral cancer cases are 11q13 (CCND1: cyclin D1, protein phosphatase 1 alpha), 7p13-22 (EGFR), 8q24 (myc), and 18q21 (bcl-2). Furthermore, several studies have been published on the rise in DNA ploidy seen in early oral lesions. Certain chromosomal areas containing tumour suppressor genes are deleted in LOH, and the loss of the gene/allele can lead to the creation of lesions[51].

In the process of gene deletion, if one allele is already inactivated by DNA damage or another hereditary mechanism, LOH may lead to tumor suppressor gene inactivation by the physical loss of chromosomal material that contains the remaining allele of a particular tumor suppressor. This type of observation is common in oral leukoplakia and many low-grade oral dysplasias. The principal genes that are frequently deleted in oral malignant lesions include CDKN2A (p16INK4A), p14 (ARF), p53, and APC. The chromosomal regions 3p14 (FHIT), 3p25 (VHL), 4q, 8p21-23, 9p21-2260, 13q14 (pRB), 17p13.1 (p53), and 18q21.1 (DCC) are the main sites of LOH in OSCC, and may be useful in the prediction of oral pre-cancer[34].

Different biomolecules (mRNA/protein) and their conformations, stabilities, and activities may be altered by point mutations of certain nucleotides, which might lead to cancer. As a result, SNPs may have the ability to identify pre-cancer. Mutations in major genes, such as p5370 and Ras, may lead to the development of oral cancer. An H-ras mutation may be introduced in early dysplasia in mouth cancer following carcinogen exposure, and it is frequently associated with oral premalignant lesions. p53 gene mutations in early oral dysplastic lesions have been reported only in tobacco users. A polymorphism of p53 at codon 7237 and the MDM2 SNP30975 indicate increased OSCC risks in some populations. Thus, this information may be used to develop predictive biomarkers for detecting oral premalignant lesions[51].

Methylation, for example, is a typical epigenetic alteration in cancer. The cytosine-phosphate guanosine (CpG) islands are abundant in some DNA regions. The 50 cytosines of CpG islands can be methylated by three known DNA methyltransferases. These CpG islands are present at the promoter regions of many tumor suppressor genes, the methylation of which may result in transcriptional silencing. CDKN2A (p16INK4A), p14ARF, CDH1, MGMT, CHFR, and death-

associated protein kinase (DAPK1) are some of the genes that have been linked to oral lesions. Another study found that in oral cancer patients, abnormal methylation of the EDNRB, KIF1A, and DCC genes is prevalent. Oral cancer has also been linked to promoter hypermethylation of mismatch repair genes (such as hMLH1 and hMSH2). When developing prediction algorithms for detecting oral PMLs, this information should be taken into account[51,54].

## RNA-Based Alterations and Biomarkers

RNA contains information on the structure of cells as well as the synthesis and regulation of proteins. The rate-limiting phase in the genesis of neoplastic alterations is the transcriptional activation or repression of certain genes, which controls protein expression levels. The regulation might be connected to transcription factors (TFs), which can stimulate gene over transcription in OSCC, increasing oncogene expression. When compared to normal tissues, oral lesions had higher levels of cyclin D1, Cdk6, and EGF mRNA expression. Upregulation of p53, EGFR, and COX-2 mRNA has also been documented in oral cancer. Leukoplakia has also been linked to lower DOC-1 levels. Overexpression of survival as a marker for early identification of oral cancer was recently reported by Lodi G et al[55].

The overexpression of TGF-α, cyclin D1 and CDK4/6 is also prevalent, in oral lesions, in addition to the stabilization of single nucleotide polymorphisms at the 3' UTR of cyclin D1. Many immunomodulatory genes (interleukins 1-a, 1-b, 8, TNF-a, and hypoxia inducible factor-1) have variants/SNPs that increase susceptibility to OSCC and oral PMLs. In many malignancies, including OSCC, miRNAs are short, non-coding RNA molecules (22 nucleotides) that bind to the 3' UTR of mRNA and influence posttranscriptional gene expression. Increased plasma miRNA (miR-31) levels have previously been linked to OSCC. Oral cancer has been linked to plasma overexpression of mir-2190,91, miR-2492, and mir-31. Additionally, oral leukoplakia has been linked to overexpression of mir-181b and mir-345. Oral pre-cancer has also been linked to low levels of miR-125b and miR-100[51].

## Protein-Based Biomarkers

Protein levels change based on physiological/pathological situations as an essential component of living organisms. These compounds may be easily identified by employing different techniques such as IHC, WB, ELISA, and immunocytochemistry to target antibodies. Many proteins are overexpressed in solid tumours, including oral cancer, including EGFR, STAT, and VEGF.

The overexpression of p53-MDM2, p63, Ki67 and maspin has been reported in association with oral premalignant lesions[56–58].

Bcl-2 overexpression has been reported in oral epithelial dysplasia, and the Bcl-2 family member Mcl-1 is also predictive of early oral lesions. Similarly, phosphatidylinositol synthase (PI synthase) overexpression has been considered to be an early marker for oral cancer, and this gene is a target of smokeless tobacco carcinogens[59].

DeltaNp63 overexpression also indicates high risk. Recent proteomic analyses revealed many pathways that may be involved in oral leukoplakia and OSCC in addition to potential diagnostic markers. Post translational modifications are common in PMLs and OSCC, including the phosphorylation of Ser, Thr or Tyr residues by various protein kinases. Phosphorylations and changes in the activities of several proteins have been established to occur in oral lesions and include pRB, some MAPK members (ERK), Akt, and STAT3. The ubiquitination of p27 by Skp2 and Cks1 has also been reported in oral cancer. Additionally, the farnesylation of various proteins, including Ras, plays a role. Oncogenic pathways that activate EGFR, Jak-STAT, PI3K-Akt-mTOR, Wnt-bcatenin, Ras-Raf-MEK-MAPK, and VEGF are of predictive value for the early detection of oral lesions with oncogenic potentials, as indicated by various biochemical and molecular studies[51,52].

## DNA Aneuploidy

Aneuploidy indicates either an unbalanced number of chromosomes (whole-chromosome aneuploidy) or unbalanced chromosomal regions due to deletions, duplications, amplifications, or translocations (segmental aneuploidy). CIN and aneuploidy, as inferred from gene expression patterns, are thought to predict clinical prognosis in a variety of human malignancies, including OSCCs. This is in line with early findings in which aneuploidy was assessed by DNA flow cytometry. This idea does not rule out the possibility that aneuploidy occurs early in premalignant lesions, such as OPMDs. In three significant but small retrospective investigations employing formalin-fixed paraffin-embedded material, DNA aneuploidy measured by imaging cytometry was used to predict malignant transformation of OPMDs. The frequency of DNA aneuploidy was statistically significantly greater in dysplastic OPMDs developing into OSCCs than in dysplastic nonprogressing OPMDs[47].

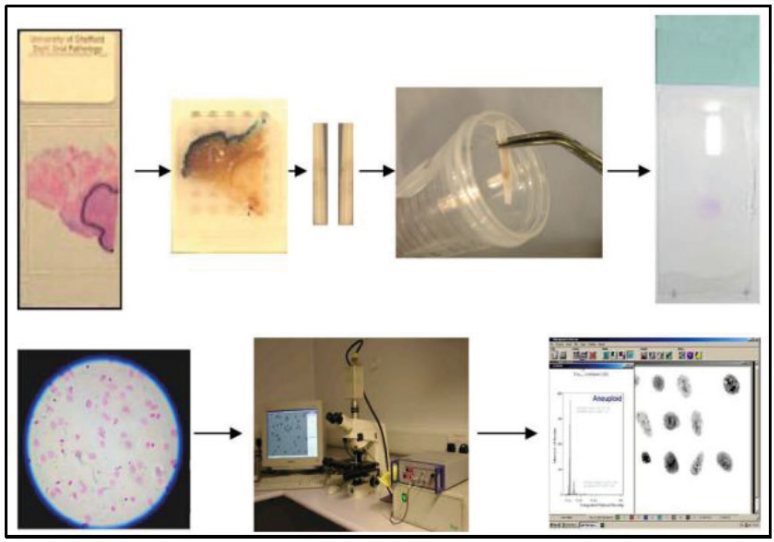


**Fig. 21: Summary of the preparation of nuclei for DNA image cytometry. The test area is selected on the H&E stained section, identified in the block and microdissected. The tissue sections are digested and a nuclear monolayer is produced. Nuclear monolayers are Feulgen–PAS stained for DNA image cytometry analysis and the results are displayed on a computer (image courtesy to Torres-Rendon et al, DNA ploidy analysis by image cytometry helps to identify oral epithelial dysplasias with a high risk of malignant progression, Oral Oncology 45 (2009) 468–473).**

Recent research suggests that DNA aneuploidy is a better predictor of malignant development in oral leukoplakias than histological dysplasia evaluation. Aneuploidy predicts malignant transformation in proliferative verrucous leukoplakia, according to a recent study. By comparing the integrated optical density (IOD) of the nuclei of interest with control nuclei, DNA image cytometry may determine the DNA content (ploidy) in tumour cell populations (usually lymphocytes). The amount of defective DNA stem lines, the polyploidization of euploid or aneuploid DNA stem lines, and the presence of uncommon cells with unusually high DNA content may all be determined using DNA image cytometry. The DNA index (DI) and the proportion of cells above 5c (5cER) are well-established techniques for interpreting image cytometry results and distinguishing between aneuploid and diploid diseases. The use of DNA image cytometry to assess ploidy as a predictive tool for the assessment of oral epithelial dysplasia has yet to be verified[52].

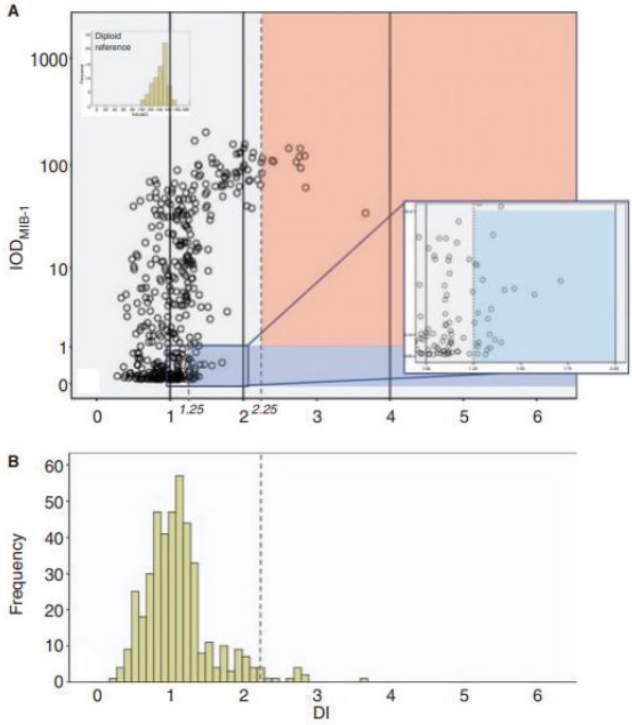



**Fig. 22: Data from a tissue section containing moderate dysplasia. (a) Scatterplot of DNA ploidy and MIB-1 expression, (b) DI histogram of the same specimen, extracted from the same data (image courtesy to Fleskens S J H M et al, Simultaneous assessment of DNA ploidy and biomarker expression in paraffin-embedded tissue sections, Histopathology; 2010, 57, 14–26).**

Genomic changes that lead to cancer may be caused by chromosomal instability or microsatellite instability. Aneuploidy, or an abnormal chromosome number, has been proposed as a valuable marker for the neoplastic evolution of premalignant lesions in several locations, including the oesophagus, skin, head and neck, and colon. When paired with phenotypic data on protein expression levels related to cell cycle progression or DNA damage response, DNA ploidy status can be extremely useful. Flow cytometry may be used to measure ploidy and protein expression in individual cells at the same time. Image cytometry, on the other hand, allows for DNA ploidy measurement using microscopy, with subsequent visual inspection of nuclei of interest. The majority of (pre)malignancy cytometric ploidy analysis is done on intact nuclei isolated from thick tissue slices. The downside of both of the foregoing approaches is that the original tissue context is gone,

making histopathological correlation difficult. Because the data is diluted by the abundance of typical euploid cells, this might lead to the overlooking of tiny aneuploid cell populations. Also, when dealing with small tissue fragments from biopsy specimens, insufficient material may be available for isolation of nuclei. Whereas the image cytometric measurement enables analysis of protein expression in situ in immunohistochemically stained tissue sections. Although ploidy analysis in such relatively thin sections is difficult[47,54]. Because nuclei are truncated in thin tissue slices (i.e. 2–5 lm), measurement accuracy is limited. As a result, the sensitivity of aneuploid cell detection is harmed. Because of nuclear overlap, the use of thick sections (>10 lm) may result in biassed sampling, with a preference for smaller nuclei and nuclei in less highly populated parts of the tissue. In practise, mathematical correction approaches developed to mitigate the effect of nuclear truncation have limited relevance. When comparing ploidy analysis of 7 m sections without integrated optical density (IOD) correction to analysis of 5 m sections with IOD correction, it was shown that ploidy analysis of 7 m sections without IOD correction is more sensitive in finding aneuploid subpopulations. According to certain research, IOD correction may potentially be damaging to the body[47,54].

The role of CIN and aneuploidy in the genesis and progression of oral premalignancy and, in particular, in their link with the concept of "oral field effect cancerization," is still relatively uncertain. The field effect theory postulates that an initial oral patch, which is not visually recognizable but characterized by stem cells sharing genetic/genomic aberrations, is converted into an expanding field with numerous aberrations. This field might become then visible as leukoplakia or erythroplakia or OPMD. OPMDs, which may be nondysplastic and dysplastic, were characterized by a relatively low rate of transformation of about 1.3% per year[32].

## Laser Capture Microdissection

Laser capture microdissection (LCM) is a technique that uses a low-energy infrared laser pulse to selectively attach cells and tissue pieces to a thermoplastic membrane. An inverted microscope, a solid-state near-infrared laser diode, a laser control unit, a joystick-controlled microscope stage with a vacuum chuck for slide immobilisation, a CCD camera, and a colour display are all part of the system. For extra laser control and picture archiving, the LCM microscope is commonly linked to a computer. The thermoplastic membrane used for cell transfer has a diameter of around 6 mm and is put on an optically transparent cover that fits on typical 0.5 ml micro centrifuge tubes for tissue processing. The cap is hung from a mechanical transport arm and put under normal pressure on the appropriate location of the dehydrated tissue segment[60].

Laser activation causes focused melting of the ethylene vinyl acetate (EVA) membrane, which has its absorption maximum around the wavelength of the laser, after visual selection of the target cells led by a positioning beam. The molten polymer spreads into the section, filling the tissue's exceedingly microscopic empty spaces. Within milliseconds, the polymer resolidifies and forms a composite with the tissue. The tissue's adherence to the activated membrane is greater than that of the glass slide, allowing for selective removal of the targeted cells. Laser pulses with a length of between 0.5 and 5 milliseconds can be repeated numerous times throughout the whole cap surface, allowing for fast cell separation. The chosen tissue pieces are collected by simply lifting the lid, and then transferred to a microcentrifuge tube containing the buVer solutions needed to isolate the molecules of interest. The LCM microscope's laser beam has a minimum diameter of 7.5 m and a maximum diameter of 30 m. Under normal operating circumstances, the region of polymer melting matches to the laser spot size almost perfectly. Because the membrane absorbs the majority of the energy, the tissue's highest temperature after laser activation is in the range of 90 °C for many milliseconds, keeping biological macromolecules intact. The infrared laser's low intensity also prevents possibly harmful photochemical effects[60,61].

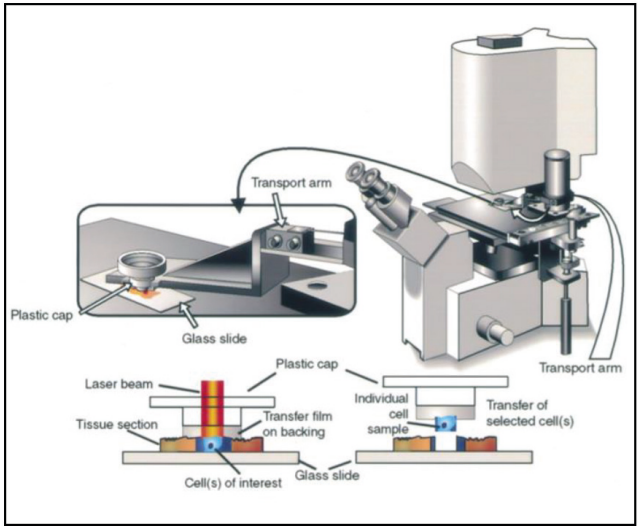



**Fig. 23: Laser capture microdissection system (image courtesy to Simone NL, Bonner RF,Gillespie JW, Emmert-Buck, Liotta LA, Laser-capture microdissection: opening the microscopic frontier to molecular analysis, Tig 1998, vol 14 no.7).**

Many tumours, including HNSCC, are heterogeneous, and include areas of connective tissues, blood vessels, and even inflammatory cells that infiltrate into the tumour mass. A novel technique of Laser Capture Microdissection (LCM) developed at the Laboratory of Pathology (National Cancer Institute) allows the procurement of pure cell populations from frozen human tissue sections (Emmert-Buck et al., 1996; Simone et al., 1998), which is important because many tumour Most crucially, microdissection enables for in-depth examination of gene expression in specific cell types[62].

## Invasive Techniques

### Biopsy

Biopsy is the removal of tissue from a living person for microscopic examination to confirm or to establish the diagnosis of a disease. There are different types of biopsy techniques.

### Surgical Biopsy

This is the surgical removal of tissue to determine the diagnosis before treatment and may be undertaken with a scalpel, biopsy punch, cutting laser, electrocautery or a wide cutting needle (core biopsy).

### Incisional Biopsy

An incisional biopsy is a technique that samples only a particular or representative part of the lesion. If the lesion is large or has different characteristics at different locations more than one area of the lesion may need to be sampled by this technique usually indicated if the area under investigation appears difficult to excise because of its extensive size (>1cm in diameter) or hazardous location or if there is suspicion of malignancy[63].

For lesions that are possibly malignant or for which the diagnosis is unknown, incisional biopsy is frequently used to determine the diagnosis prior to therapy. In a wedge pattern, biopsies of representative sections of the lesion should be taken. The biopsy location should be in a region with the most tissue alterations. It's best to avoid necrotic tissue because it's useless for diagnosis. To include normal tissue, the sample should be obtained from the margin of the lesion. It's important to make sure there's enough aberrant tissue in the mix. Because surface alterations may be quite different from those deeper in the tissue, it is preferable to take a deep, narrow biopsy rather than a broad, shallow one. Except for pigmented or vascular lesions, fast developing lesions with ill-defined boundaries, which need margins of 5 mm normal tissue, a margin of normal tissue of at least 2–3 mm is necessary[63,64].

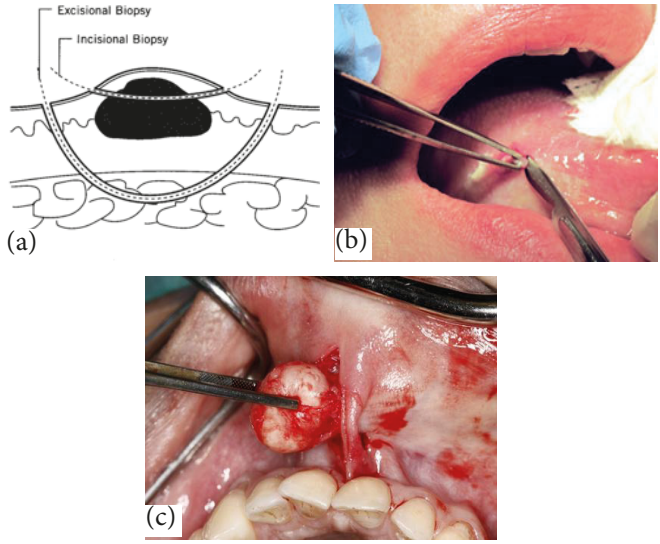



**Fig. 24: (a) Diagrammatic representation of incisional and excisional biopsy. (b) Incisional biopsy performed on right lateral border of tongue (c) Excisional biopsy obtained from upper lip (image courtesy to https://dental.washington.edu/oral-pathology/case-of-the-month-archives/com-december-2010/, J Can Dent Assoc 2012;78:c75)**

## Excisional Biopsy

This is useful for excising simple mucosal and soft tissue lesions where diagnosis and curative intent can be achieved simultaneously. The entire lesion, along with 2–3 mm of normal appearing surrounding tissue is excised. Excisional biopsy should be used for smaller lesions (less than 1 cm in diameter) that seem benign on clinical inspection. Excisional biopsy is the best treatment for any lesion that can be removed entirely without mutilating the patient. All pigmented and minor vascular lesions should be completely eliminated. This method should only be performed if the lesion can be removed without causing harm to important structures. The level of dysplasia varies at different areas across the lesion, hence excision of OPMD lesions is done following a first incisional biopsy and histological evaluation[63,64].

## Punch Biopsy

It can be incisional or excisional, but it's commonly a variation of an incisional biopsy, in which a piece of the lesion is removed with a specific punch type forceps.

The surgical device files off tiny pieces of tissue from inaccessible lesions or huge lesions where excision is contraindicated in this procedure. Because this equipment frequently causes lesion distortion, this procedure is rarely employed[63].

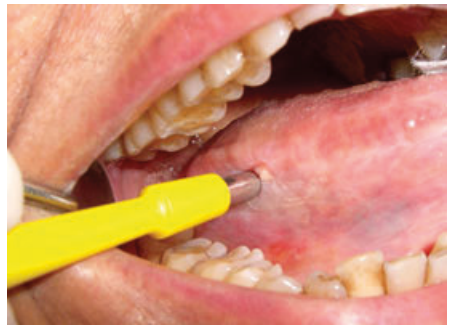


**Fig. 25: Punch biopsy performed on the right lateral border of tongue (image courtesy to J Can Dent Assoc 2012;78:c75)**

The use of the punch biopsy for definitive surgical excision of oral mucosal lesions is limited and sizes available for biopsy punches range from 2 to 6 m m in diameter. The technique is contraindicated for the removal of suspected malignant lesions. The oral mucosal punch biopsy technique is used most often for incisional biopsies for diagnosis before treatment[65].

The technique can be used for detection of oral mucosal malignancies such as squamous cell carcinoma as well as premalignant lesions such as leukoplakias and other mucosal abnormalities that may require multiple biopsies[65].

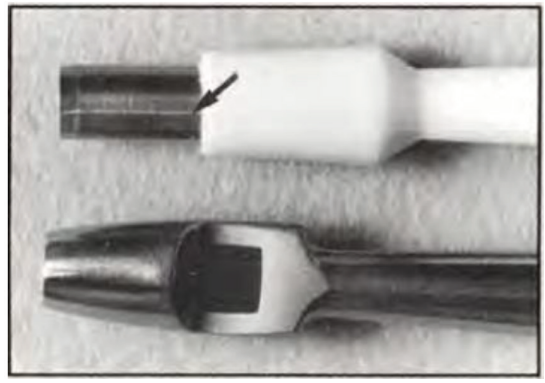


**Fig. 26: A disposable plastic-handled punch on top and a Keyes metal punch shown on bottom (image courtesy Lynch DP , Morris LF, The oral mucosal punch biopsy: indications and technique, JADA; 1990, Vol. 121, 145-149).**

## Types of Biopsy Punches

A number of types of reusable biopsy punches are available. The Keyes biopsy punches were originally developed for dermatologie use, but it can also be used for oral mucosal lesions. These handheld punches range in size from 1.0–12.0 mm in increments of 0.25–0.50 mm. Similar biopsy punches are available for use with a belt-driven engine at speeds of 250–36,000 rpm. In most cases, these belt-driven punches are in appropriate for oral mucosa. Both the Keyes and the belt-driven punches are made of surgical stainless steel[65].

Disposable biopsy punches are sterile, prepared devices that come in a range of sizes and are less costly than reusable metal punches. They have a steady sharpness and a good tactile sense. The reusable metal punch weighs 25 g, whereas the disposable plastic punch weighs around 2 g. The exterior blade bevel is particularly useful in approximating the depth of the surgical incision. The plastic handle may be shortened for better access to difficult areas. The only disadvantage to disposable biopsy punches is that the blades are machine-rolled with a detectable seam. Occasionally, this seam will catch on mucosa and cause some minor distortion of the specimen[65].

## Technique

The biopsy punch can be held between the thumb and index finger once the biopsy site has been chosen, numbed, and gently wiped with sterile gauze. The blade's edge is put on the oral mucosa, and the punch is spun back and forth between the fingers with moderate pressure until the exterior bevel is no longer visible. The surgical incision should reach into the connective tissue at this point, but not the deep lamina propria. If there is some uncertainty about the adequacy of the depth of the initial incision, then the punch can be withdrawn, the lesion gently blotted, and the need for additional depth assessed. If necessary, the punch can be replaced in the original incision and the procedure continued until an appropriate depth is reached[65].

## Fine Needle Aspiration Cytology (FNAC)

It is minimally invasive and well tolerated. Ultrasound-guided FNAC increases diagnostic accuracy by enabling avoidance of necrotic or cystic regions and the targeting of high-yield areas of the lesion for tissue extraction. The operator can also validate the location of the needle tip in the lesion using ultrasound guidance. The main drawback is that FNAC delivers differently grouped cells with no tissue

architecture or supply stroma, which is crucial for proper diagnosis in a biopsy sample. One of the problems with FNAC is that it might cause changes in tissue, which can make it difficult to diagnose during the final histological examination. This is especially true of glandular tissue when assessing salivary glands[64].

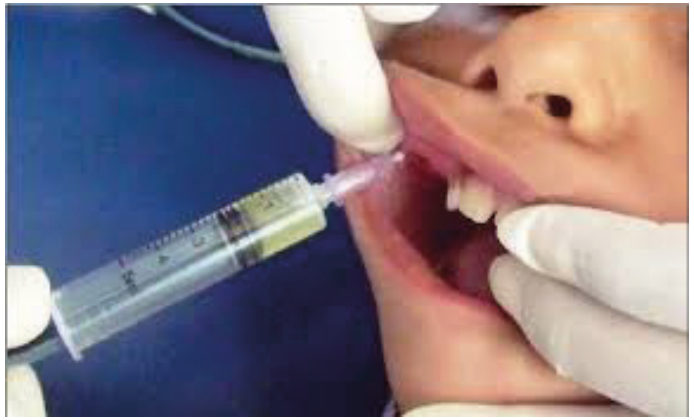


**Fig. 27: Fine needle aspiration cytology (image courtesy to https://pds.cdeworld.com/courses/20970-oral-pathology-techniques-for-detection-and-differential-diagnosis).**

## Exfoliative Cytology

The microscopic inspection of shed cells from an epithelial surface is known as exfoliative cytology. Its primary use has been in the detection of uterine cervix dysplasia and cancer. Exfoliative cytology is a quick, non-invasive process that can be used to detect oral squamous cell cancer (SCC)[66].

Indications for oral exfoliative cytology includes the following[66]:

1. Periodic review of 'high risk' patients.
2. Periodic review of oral premalignant lesions.
3. For the determination of a suitable biopsy site.
4. In cases where biopsy is contraindicated on medical grounds.
5. Unavailability of embedding and sectioning technology.
6. Periodic review of oral cancer patients.
7. Screening family members of patients with oral cancer.
8. Population screening for oral cancer.

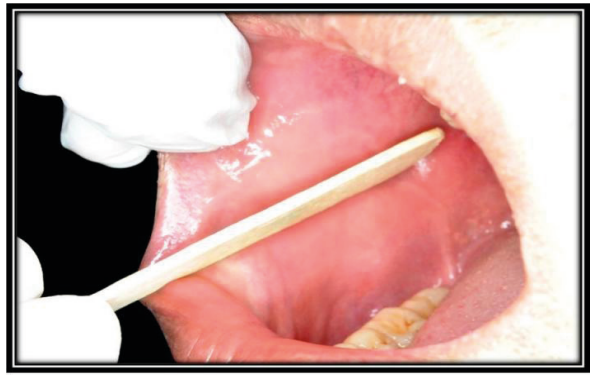


**Fig. 28: Sample collection being done for exfoliative cytology using wooden spatula (image courtesy to** https://jcdr.net/article_ **fulltext.asp?issn= 0973709x&year= 2016 & volume=10&issue=7&page=ZC05&issn=0973-709x&id=8072).**

A collection instrument (swab, spatula, and brush) is put against the mucosal surface and turned to collect the cells in this procedure. The next step is to distribute the cells over a glass slide to make a smear. The slide is reviewed by a pathologist after fixation and papanicolaou staining. The results must be interpreted by a cytopathologist who is well-versed in the subject. A cytobrush is sometimes used to gather cells[66].

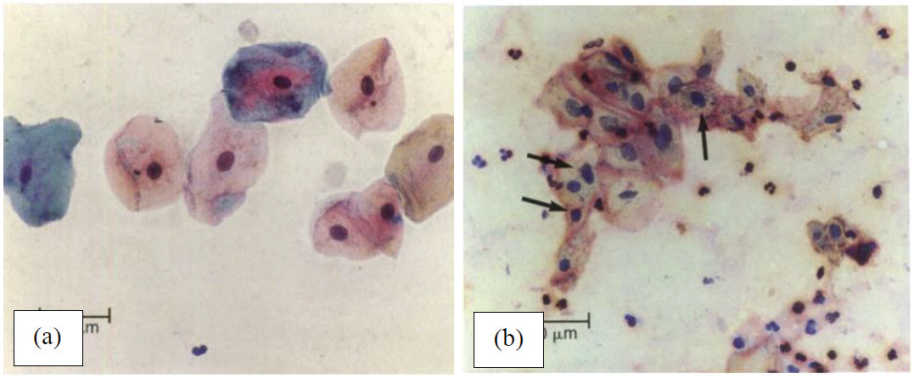



**Fig. 29: Appearance of epithelial squames in smears obtained by exfoliative cytology stained with Papanicolaou EA50 solution a) in normal buccal mucosa b)- in oral squamous cell carcinoma showing particularly the altered nuclear: cytoplasmic ratio and the nuclear hyperchromatism and pleomorphism (arrows), numerous polymorphonuclear leukocytes are also present. (image courtesy to ref Sugerman PB, Savage NW, Exfoliative cytology in clinical oral pathology, Australian Dental Journal 1996;41:2).**

## Liquid Based Cytology (LBC):

LBC is a cytology approach that can compensate for many of the drawbacks of traditional exfoliation cytology. A suspension of cells is obtained instead of a single smear, and multiple slides can be created. In LBC, the sample collector is submerged in a tube containing preservative solution, which quickly fixes the cells. The cells form a thin high cellular restricted zone on the glass slide when the tube is placed on a centrifugal force, which the pathologist may simply examine. Many standard cytology artefacts are absent with the LBC approach[2,67].

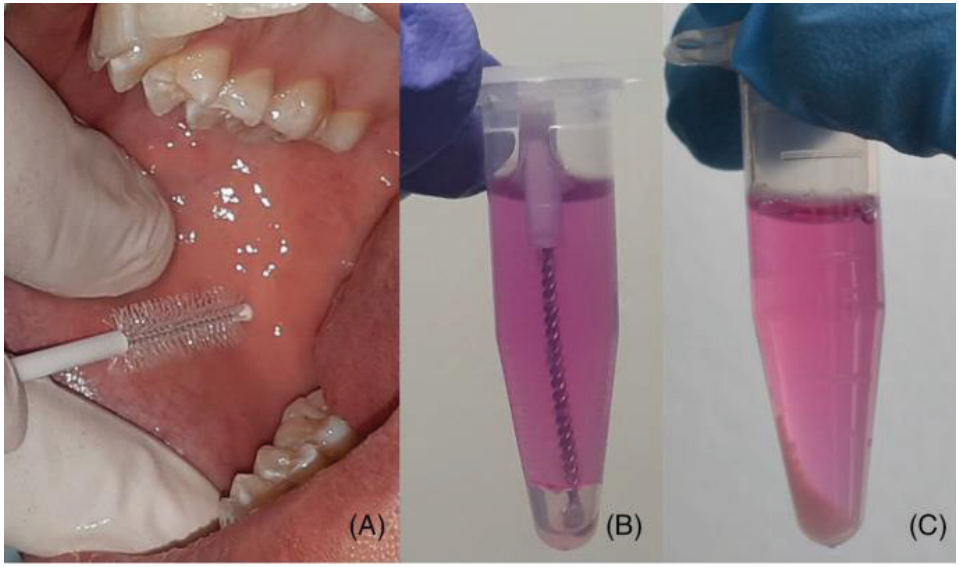



**Fig. 30: Liquid based cytology (image courtesy to Osorio-Osorno YA, Toro DA, Arango JC, Parada-Sanchez, Optimized liquid-based cytology for the cellular and molecular analysis of oral keratinocytes: A promising diagnostic tool ,Diagnostic Cytopathology. 2020;1–9).**

Liquid-based cytology, which is mostly used for cervical smear examinations, has shown promise in terms of improving sample preparation quality (since there were no cellular artefacts, it was possible to analyse all the cells collected). Furthermore, it produces reliable findings that are simpler to standardise, and the leftover material after cytological inspection may be employed for additional analysis. However, liquid-based cytology has only been employed in the identification of possibly malignant lesions in the mouth on a few occasions[68].

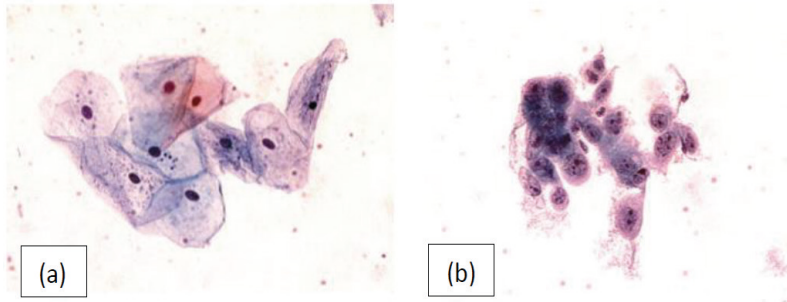



**Fig. 31: a) Normal squamous epithelial cells obtained with liquid-based technique b) Dysplastic squamous epithelial cells obtained with liquid-based technique (image courtesy to R. Navone et al, The impact of liquid-based oral cytology on the diagnosis of oral squamous dysplasia and carcinoma, Cytopathology 2007, 18, 356–360).**

## Oral Brush Biopsy

Oral brush biopsy may be used as an adjunct to incisional biopsy for lesions where there is uncertainty in the aetiology, continuing monitoring, recurrence or difficultly to excise surgically. It can also be used in resource-challenged areas. Various tools can be used from a Cytobrush, baby toothbrush, wooden spatula etc. to collect cells[64].

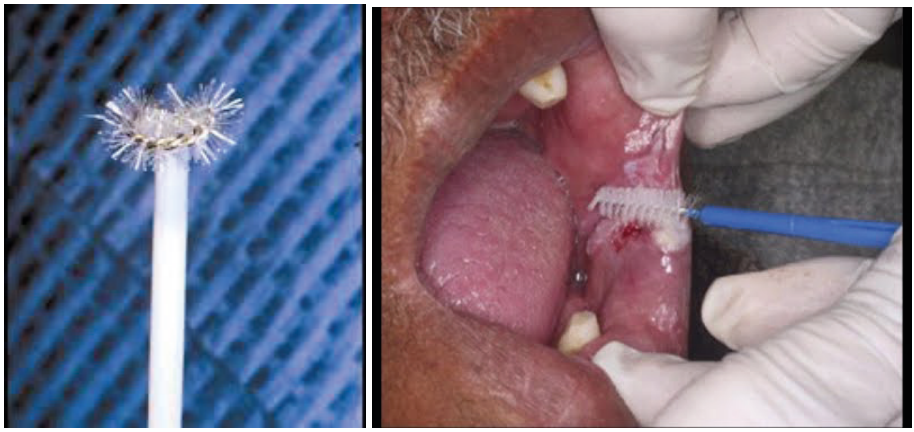


**Fig. 32: Oral CDx brush (image courtesy to Rutenberg M, Educational Presentation Educational Presentation to the ECCC to the ECCC Oral CDx Oral CDx Brush Biopsy Brush Biopsy - Technique and Indications Technique and Indications, CDx Laboratories; 2008).**

Brush biopsy (Oral CDX) was developed in 1999 and has become popular in dental practice today. In the past decades, adjunctive technique has facilitated the early detection of oral premalignant and malignant lesions (OPML)[4]. The oral brush biopsy, also known as the Oral CDx Brush Test system, is a technique for obtaining a trans-epithelial sample of cells from a mucosal lesion that includes representation of the epithelium's superficial, intermediate, and parabasal/basal layers. This test was created to look at mucosal abnormalities that would not ordinarily be exposed to biopsy due to low-risk clinical characteristics[1]. Oral CDx is useful in the assessment of dysplastic changes in various suspected lesions especially in oral cancer. As majority of oral cancers are squamous cell carcinomas, Cytological study of oral cells is a relatively inexpensive, simple, non-invasive and also risk-free technique which is well accepted by the patient and medical practitioner today[1].

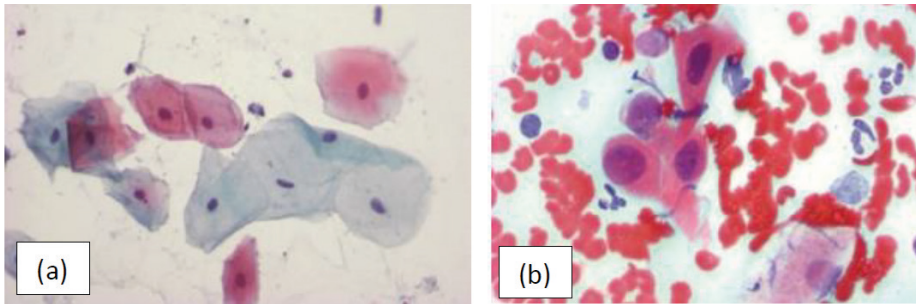



**Fig. 33: (a) Normal, (b) dysplastic, and (c) malignant oral squamous cells from brush biopsy and Papanicolaou stained (image courtesy to Bocking I et al, Review Article Role of Brush Biopsy and DNA Cytometry for Prevention, Diagnosis, Therapy, and Follow up Care of Oral Cancer A, Journal of Oncology; 2011).**

Oral cells are collected using a cytobrush, a specifically developed brush that is a non-lacerational instrument for collecting epithelial cells. Samples are then fixed onto a glass slide, stained with a modified Papanicolaou test, and microscopically analysed using a computer-based imaging system. When cellular morphology is highly suspect for epithelial dysplasia or cancer, or when aberrant epithelial alterations are of unknown diagnostic significance, the results are characterised as "positive" or "atypical." When no anomalies are discovered, the results are classified as negative. When an abnormal result is recorded, a scalpel biopsy must be performed, hence the test is considered an intermediate diagnostic step (atypical or positive)[1].

## Frozen Sections

Frozen section technique is a method of freezing tissues as a means of hardening them to allow the preparation of thin sections. This technique allows a stained slide to be examined within 10 minutes of taking the specimen. The tissue is sent fresh to the laboratory to be frozen by immersion in liquid nitrogen (–196°C) or dry ice (–78°C), very cold to ensure freezing is near instantaneous and does not allow time for ice crystals to form in the tissue. A section is then sliced and stained on a refrigerated microtome.

The equipment for frozen sections is often in the theatre suite to speed the process even further. Frozen section is more commonly utilised to determine whether excision margins are cancer-free than to establish an initial diagnosis. Techniques such as fine needle aspiration biopsy or a routine specimen with specific quick laboratory processing are typically preferred if a speedy diagnosis is necessary in other conditions. The principal indication of performing a frozen section is to obtain a definite histologic diagnosis on the basis of which a surgical decision will be made. It is also important in assessing the extend of local or distant metastasis of malignant tumors and in determining the adequacy of resection before the wound is closed[69,70].

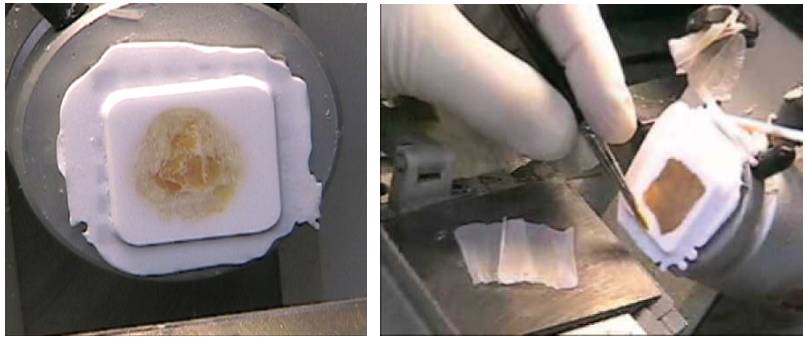


**Fig. 34: Frozen section technique (image courtesy to Remsen KA et al, Reliability of frozen section diagnosis in head and neck neoplasm, Laryngoscope 94; 1984).**

❑

# Conclusion

To conclude, regardless of all the type of oral cancer, it is still unknown what really causes its development. Recent research suggest that tobacco use is a primary cause of oral cavity and oropharyngeal cancer, with smokers having a higher risk of developing the disease than non-smokers. Carcinogens found in high concentration in tobacco and tobacco products are the major cause of cancer in the lungs, oesophagus, and other organs. Oral cancer screening and early diagnosis with the numerous diagnostic tools listed below reduce the risk of morbidity and death associated with the disease. In the last few years, there has been a significant growth in the creation of various prospective oral cancer screening procedures, and many researchers are still looking for better and faster ways to diagnose these life-threatening tumours[3,4].

There are many diagnostic aids for early detection of oral cancer. Yet the gold standard of oral cancer diagnosis is surgical biopsy, which can be performed by a trained dentist/physician. Diagnostic aids can be used in different situations specially when a surgical biopsy is not indicated and can help the clinician to:

1. Choose the best site for biopsy
2. Follow up a patient with a premalignant lesion
3. Screen for oral cancer in high-risk patients or high risk sites of oral cavity (e.g. ventral side of tongue, floor of the mouth etc.)
4. Make a preliminary diagnosis when there is a systemic contraindication for surgical biopsy 5. Differentiation of pseudoepitheliomatous hyperplasia from a real malignancy[6,9].

It is apparent that early diagnosis of cancer and pre-cancerous lesions by screening has the potential to lower the disease's morbidity and mortality. Advances in saliva-based oral diagnostic and optical biopsy technologies offer promising avenues for the future development of more effective non-invasive approaches for diagnosing OSCC that may be performed clinically in primary care settings[6].

The ability of dentists to diagnose oral cancer in its precancerous stage plays a crucial role in preventing it from progressing to later stages. In order to enhance early diagnosis, health-care providers must have a greater understanding of oral

cancer, its risk factors, and the most prevalent oral precancerous disorders. Future research can also be directed towards establishing appropriate clinical practice standards for early detection exams. Currently, the new innovative visual-based techniques show promising results, but lack strong evidence to support their effectiveness in early detection. Their utilization in clinic practice is still anecdotal. Limitations that hinder their wide use include lack of methodologically sound clinical trials, their correlation with histological alterations and the impact these techniques have on a patient's survival and risk of disease recurrence[7,8].

Oral cancer morbidity and mortality have been suggested to be reduced by screening and early identification in at-risk groups. Visual identification of premalignant oral lesions, on the other hand, has remained a challenge all over the world. Oral cancer and precancer lesions are frequently modest, and clinical features such as ulceration, induration, discomfort, or accompanying cervical lymphadenopathy are only seen in advanced instances. Besides their clinical subtle, premalignant lesions are highly heterogeneous in their presentation and may mimic a variety of common benign or reactive conditions. Furthermore, there is a growing realization that some premalignant and early cancerous lesions are not readily detectable to the naked eye. As such, additional screening aids for oral cancer are desperately needed[7,16].

Fortunately, there has been a dramatic increase in the development of potential oral cancer screening or case finding tools in the last decade. Each of them may hold promise in selected clinical settings. Many studies that have been performed using these diagnostic devices also suffer from the fact that they are being employed in a ''case-finding'' fashion, rather than as true screening tools. That is they are being used to aid in the diagnosis of a lesion that has already been identified by the naked eye. Several of the technologies (ViziLite Plus, MicroLux DL, toluidine blue and VELscope) may be useful in a true screening fashion. However, investigations to evaluate their value in this situation are expected to begin in the near future. Regardless of the findings of these research, new technology and its accompanying marketing have definitely had a positive influence on dentistry by pushing professionals to do more thorough oral cancer tests on a regular basis. Until recently, surveys had consistently demonstrated a limited understanding of proper oral cancer screening and diagnosis among the dental community. Preliminary results from a recent oral cancer awareness campaign in the United States, however, suggest that intensive, well-designed and prolonged attempts to educate the dental community as well as their patients may increase overall

awareness about the disease. To capitalize on this increasing awareness, well-designed clinical studies are needed to help dental scientists and clinicians assess the various new and evolving diagnostic aids for oral cancer and precancerous lesions. Validity, comparability to the gold standard of histopathologic analysis, suitability of patient population, use of appropriate study doctors, specificity, and replication potential must all be addressed by scientific publications and their readers. Oral cancer identification and diagnosis have long been a big concern for dental and medical professionals all over the world. When paired with better public awareness of oral cancer in general, strong diagnostic techniques that allow clinicians to locate tumours invisible by traditional examination processes should help more patients become long-term survivors of this challenging illness[7,16].

❑

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# Abbreviations

| | |
|---|---|
| AIDS | : Acquired immunodeficiency syndrome |
| AJCC | : American Joint Committee on Cancer |
| ALA | : Aminolevulinic acid |
| A/LCI | : Angle-resolved low coherence interferometry |
| CCD | : Charge coupled device |
| CK | : Cytokeratins |
| COE | : Conventional oral examination |
| COX | : Cyclooxygenase |
| CpG | : Cytosine-phosphateguanosine |
| CRM | : Confocal reflectance microscopy |
| DAPK | : Death-associated protein kinase |
| DI | : DNA Index |
| DNA | : Deoxyribonucleic acid |
| EBV | : Epstein Barr Virus |
| EGFR | : Epidermal growth factor receptor |
| ELISA | : Enzyme-linked immunosorbent assay |
| ESS | : Elastic scattering spectroscopy |
| EVA | : Ethylene vinyl acetate |
| FAD | : Flavin adenine dinucleotide |
| FNAC | : Fine Needle Aspiration Cytology |
| FS | : Fragile Sites |
| HCV | : Hepatitis C virus |
| HPV | : Human Papilloma Virus |
| HSV | : Herpes simplex virus |
| IARC | : International Agency for Research on Cancer |

| | |
|---|---|
| IHC | : Immuno hisochemistry |
| IL | : Interleukin |
| INHANCE | : International Head and Neck Cancer Epidemiology Consortium |
| IOD | : Integrated optical density |
| ISH | : In situ hybridization |
| LBC | : Liquid based cytology |
| LCM | : Laser capture microdissection |
| LIF | : Laser-induced fluorescence |
| LOH | : Loss of heterozygosity |
| MAPK | : Mitogen Activated Kinase |
| MMP | : Matrix metalloproteinases |
| MN | : Micronucleus |
| MNP | : Magnetic nanoparticles |
| NADH | : Nicotinamide Adenine Dinucleotide Hydrogen |
| NBC | : Nano biochip |
| NMR | : Nuclear magnetic resonance spectroscopy |
| OCT | : Optical coherence tomography |
| OED | : Oral epithelial dysplasia |
| OLP | : Oral lichen planus |
| OPMD | : Oral premalignant disorders |
| OPL | : Oral pre malignant lesions |
| OSCC | : Oral squamous cell carcinoma |
| PCR | : Polymerase chain reaction |
| PG | : Prostaglandin |
| RES | : Reticuloendothelial system |
| RNA | : Ribonucleic acid |
| SCC | : Squamous cell carcinoma |

| | |
|---|---|
| SCCHN | : Squamous cell carcinoma of Head and Neck |
| TB | : Toluidine blue |
| TF | : Transcription factors |
| TIRFM | : Total internal reflection fluorescence microscopy |
| TNF | : Tumor necrosis factor |
| TNM | : Tumour, node and metastasis |
| UICC | : Union Internationale Contre le Cancer (International Union Against Cancer) |
| UV | : Ultra violet |
| VEGF | : Vascular endothelial growth factor |
| WB | : Western blot |
| WHO | : World Health Organization |

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# Handbook of Diagnosis for Oral Cancer-Basics to Advanced

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## About The Book

Oral cancer is a global challenge with high burden on the society and family. Increasing incidence of oral cancer to be reduced by awareness to the general public. There is need for the health workers around the world also to be updated on recent advances in oral cancer. Keeping the above reasons in mind, the book comprising of various issues pertaining to oral cancer is written. When it comes to oral cancer management, two important issues have to be considered – diagnosis and management. Diagnosis of oral cancer has come a big way from basics to advanced non invasive and invasive methods. Early diagnosis of oral cancer plays a vital role in the management of oral cancer. It is the accurate or precise diagnosis which will help the clinician to make appropriate decisions on the management protocol. First four chapters helps the clinician to understand etiology or risk factors, various ways to classify oral cancer and its clinical presentation. Staging of oral cancer is a crucial step in diagnosis as it reports to the oncologist. On the basis of staging, surgeon will decide the treatment protocol. Decision is made on the customized treatment protocol for each of the patients. Final chapters comprise of numerous methods of diagnosis. It deals with several methods from basic to advance. It may be chosen based on the circumstances and availability of materials. Hence this book will be useful to clinicians and researchers in the field of Oral medicine. Undergraduates and postgraduate information and knowledge will be enhanced. We all look forward to future developments in diagnosis paradigms that will lead to even better outcomes for patients with oral cancers, and this textbook will serve as an important point of reference for these future advancements.

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ISBN 978-93-91208-71-4