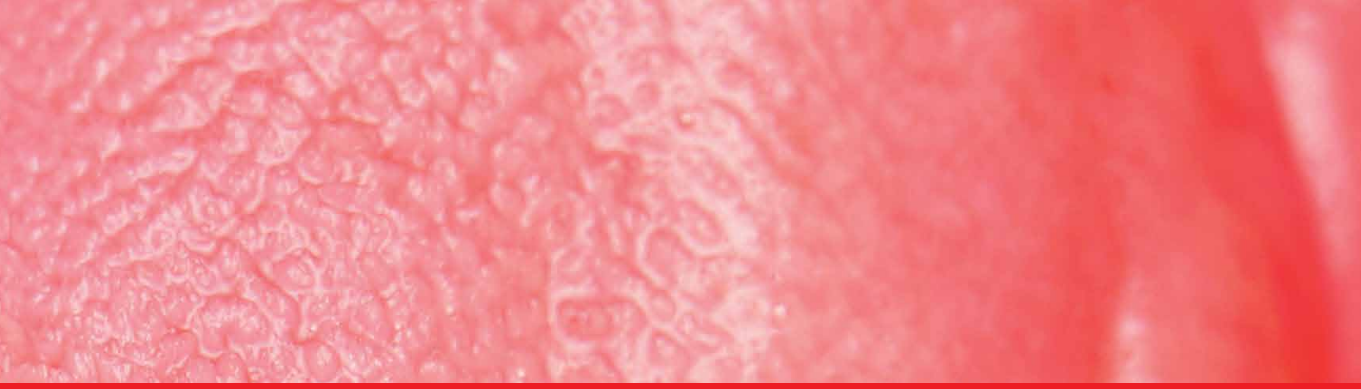

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Oral Cancer

Current Concepts and Future Perspectives

Edited by Gokul Sridharan

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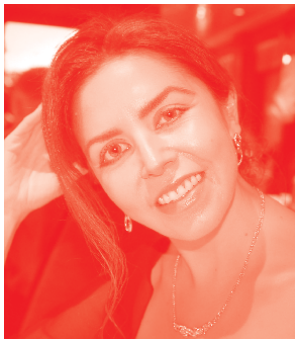

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Meet the editor

Dr. Gokul Sridharan is currently an associate professor in the Department of Oral Pathology and Microbiology, YMT Dental College and Hospital, Navi Mumbai. He obtained a Ph.D. for his work titled “Salivary and serum metabolomics in oral leukoplakia and oral squamous cell carcinoma.” His fields of interest include oral pre-cancer, oral cancer, salivary diagnostics, metabolomics, and oxidative stress. He has several scientific publications to his credit and actively contributes as a peer reviewer to numerous journals. He is an active member of the editorial boards of several journals of repute. Dr. Sridharan has undergone training and is a qualified diploma holder in medical law and ethics and is also certified in tobacco cessation and control.

Contents

Preface

"The eyes do not see what the mind does not know"

Oral cancer is one of the most prevalent diseases worldwide with significant morbidity and mortality. The term oral cancer is a broad term that encompasses malignancies arising from epithelial tissue, mesenchymal tissue, and salivary glands, to name a few. The complex nature of the head and neck anatomy poses a challenge to understanding the various malignancies occurring in this region and thereby challenging the treatment and prognosis of oral cancer. Despite technological advancements in the 21st century, there is little improvement in the five-year survival rate and post-treatment quality of life. The current challenge facing the scientific community and clinicians is to identify means of early diagnosis of oral cancers and improve treatment strategies that could minimize morbidity and improve quality of life. Various technologies for early diagnosis such as identification of tumor biomarkers in saliva and serum, determination of genetic and molecular alterations to predict the behavior and prognosis of oral cancer, implementation of newer methods of treatment such as targeted therapy, immunotherapy, and so on, are being evaluated constantly for clinical application in oral cancer therapy. This book reviews the current concepts concerning oral cancer as well as future perspectives directed towards understanding scientific advancements in managing the disease.

Chapters cover a range of topics such as prevention of oral cancer in hookah smokers, novel methods of cancer detection using salivary exosomes, the role of cytopathology in intra-oral salivary gland tumors, precision personalized medicine, sentinel lymph node biopsy, and applications of various molecular markers and radiotherapy in the treatment of oral cancers.

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Chapter 1

Oral Cancer: Epidemiology, Prevention, Early Detection, and Treatment

Ali Khani Jeihooni and Fatemeh Jafari

Abstract

One of the most common types of cancer is head and neck cancer. Head and neck cancers are the sixth most common cancer worldwide and the most common cancer in developing countries. Oral cancer, which is a subset of head and neck cancers, refers to any cancerous growth in the oral cavity. Risk factors for oral cancer include age, malnutrition, genetic factors, family history, X-rays, papilloma virus, alcohol, smoking, tobacco, which three last are the strongest risk factors. The destructive link between tobacco products and human cancers stems from a powerful combination of two factors - nicotine and carcinogens. The highest incidence of tobacco related oral cancer is seen in low and middle income countries. The chance of curing oral cancers increases if they are diagnosed and treated early. At least three-quarters of all oral cancers can be prevented by quitting smoking and drinking alcohol. Screening programs can be valuable in patients from high-risk groups (smokers and alcoholics) or in patients with a previous diagnosis of cancer outside the head and neck.

Keywords: head and neck cancer, oral cancer, smoking, tobacco, screening

1. Introduction

In the present century, the rapid growth of non-communicable diseases is considered as a serious health challenge that threatens the socio-economic development of communities and people's health [1]. The most common types of non-communicable diseases are cardiovascular disease, diabetes, chronic respiratory disease and cancer [2]. Cancers have been known as life-threatening conditions all over the world [3] and recognized as one of the most significant reasons of death around the world and every year, more than 10 million infections and 6 million deaths caused by cancers are reported [4].

One of the most common types of cancer is head and neck cancer [5]. Head and neck cancers are the sixth most common cancer worldwide and the most common cancer in developing countries. While head and neck cancers are one of the most common cancers in South and Southeast Asia, they account for only 1% -4% of all cancers in the Western world [6].

Oral cancer, which is a subset of head and neck cancers, refers to any cancerous growth in the oral cavity. This cancer includes tumors of the lips, tongue, cheeks, gum, floor of the mouth, soft and hard palate, sinuses, tonsils, salivary glands and

throat that can be fatal if left untreated. More than 90% of types of oral cancers originate in the squamous cells that line the inside of the mouth. When the growth of these cells gets out of control, it causes a cancer called squamous cell carcinoma or squamous cell carcinoma. Other types of oral cancers, such as partial malignancies of the salivary glands, sarcomas, odontogenic malignancies, melanoma, and lymphoma, make up less than 10% of oral cancers [7] and approximately 1% of metastatic cancers are lung, breast, prostate and kidney [8]. Squamous cell carcinoma can have various levels of differentiation and often give rise to node metastases. Lymphatic spreading into the neck is directly related to the T stage as well as the depth of invasion and tumor thickness [9].

2. Clinical forms

Tumors may appear in various forms of ulcers, prominent fungal masses, papillary, wart-like, white and red plaques, or a combination of both. Many primary

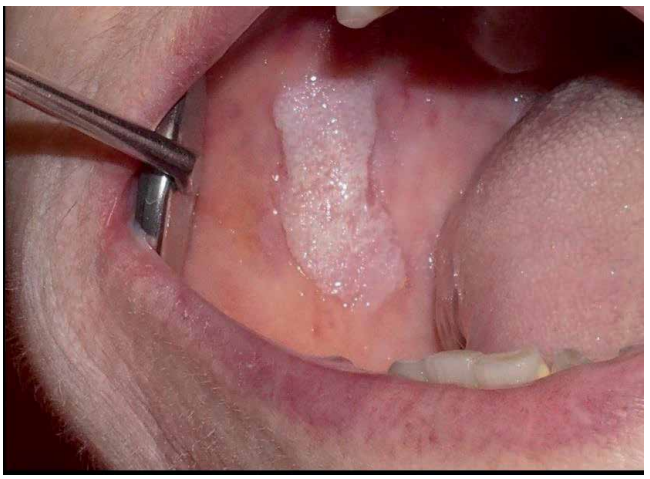

Figure 1.
Verrucous leukoplakia in the right buccal surface of a 72-year-old woman.

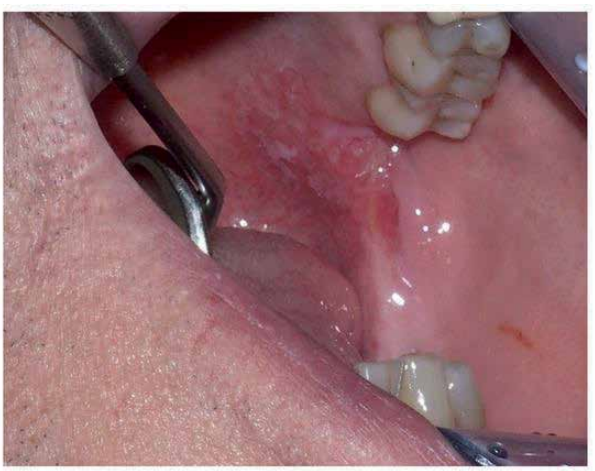

Figure 2.
Erythroplasia of the posterior hard palate, on histopathological examination in a 61-year-old man.

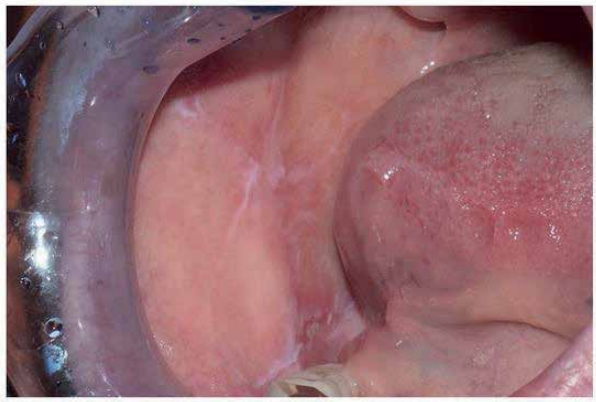

Figure 3.
White reticular striae on the right cheek mucosa of a 52-year-old man with oral lichen planus.

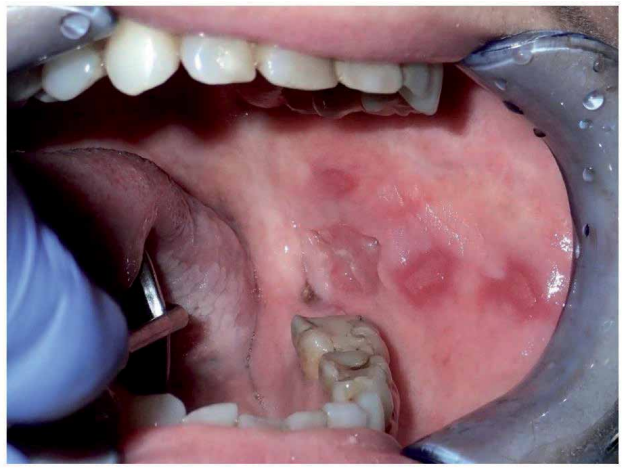

Figure 4.
Multiple areas of erythroplakia with ulceration in the left buccal mucosa because of chronic traumatic ulcer from self-biting in a 53-year-old woman.

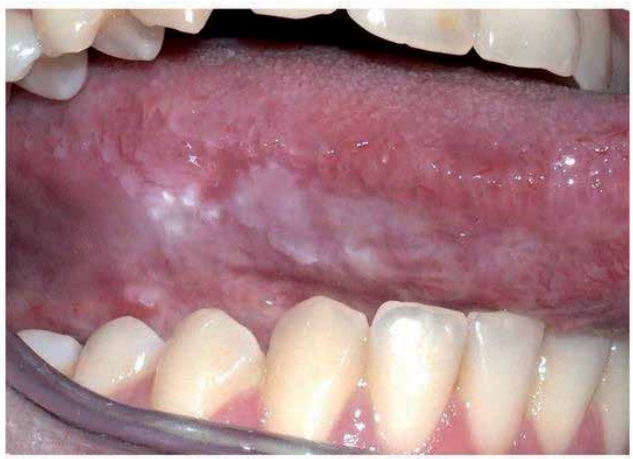

Figure 5.
Leukoplakia in the right border of the tongue with severe dysplasia in a 25-year-old woman.

cancers of the mouth are asymptomatic, while advanced lesions are often ulcerative and have prominent, hard margins. Pain is often absent in the later stages of the disease [8]. Potentially malignant oral epithelial lesions (PMOEL) are a group of oral diseases that may exist before the onset of oral squamous cell carcinoma (OSCC) and include a group of clinically oral mucosal lesions such as leukoplakia, erythroplakia, submucosal fibrosis, and lichen planus. However, most PMOELs do not progress to cancer (**Figures 1**–**5**) [10].

3. The place of conflict

The most common site of oral cancer is the lip. Lip cancer often occurs in light-skinned older men and most often affects the lower lip. Risk factors for lip cancer include sun exposure, certain occupations such as agriculture, socioeconomic status, smoking and pipe. Inside the mouth, the high-risk sites for cancer are the abdominal surface, the posterior sides of the tongue, the floor of the mouth, and the soft palate. Tongue cancer is the most common malignancy in the mouth. Tongue cancer is more common in men in their sixth and seventh decades of life. Tongue lesions are often aggressive. The second most common intraoral site for cancerous changes is the floor of the mouth. Tumors in this area occur in older men, especially in smokers and alcoholics. Involvement of the cheeks and gums is also common, especially in areas where there are certain habits such as chewing tobacco [8].

4. Risk factors

Oral cancer usually occurs in people over the age of 40 with an average age of 60, and their risk increases with age. It affects most men, but may increase as women smoke. Racially, black Americans are at higher risk for oral and throat cancer than whites. This increase in risk seems to be due to the influence of environmental factors, because the role of genetic factors in its occurrence has not been determined. Patients who smoke or chew a lot of tobacco and people who drink a lot of alcohol are at higher risk for oral cancer. Exposure to UV rays in people who stay in the sun for long periods of time is more likely to develop lip cancer. This is why the incidence of lip cancer is high in Australia. Other factors such as immunosuppression (such as AIDS and organ transplantation), viral papillomavirus infection (especially type 16, which accounts for 63% of new cases of oral cancer), Plummer–Vinson syndrome, and vitamin A deficiency also increase the risk of oral and pharyngeal cancer. The prevalence of HPV-related oral and pharyngeal cancers (mainly HPV type 16) has been increasing in North America and Northern Europe [11]. Other factors, including arsenic compounds used to treat syphilis, nutritional deficiencies, exposure to compounds such as wood and metal particles, and Candida infection, play a lesser role in cancer [8].

4.1 Hookah

In recent years, hookah has spread to Europe and the United States. In most countries, the increasing trend of hookah consumption is due to the increase in fruit and flavored tobacco products [12]. In a study conducted in Iran, the prevalence of hookah use among young people was reported to be 33.9%, which is higher than the number of people who smoke [13]. The side effects of hookah are many because the smoke produced from tobacco is composed of 4000 different chemicals and more than 40 carcinogens [14]. Tobacco smoke and hookah use are the most

important risk factors for oral cancers and dysplastic lesions [3]. Cigarettes or other tobacco-related compounds are associated with about 75% of oral cancers. Tobacco contains more than 60 known carcinogens. The use of tobacco, whether in a smoky or chewable form alone, and especially with heavy alcohol consumption, is a very important risk factor for oral cancer. Smokers are 7 times more likely to develop oral cancer than non-smokers. The relative risk of developing cancer in people who consume a lot of alcohol is 6 times higher, and this risk is 38 times higher for patients who use alcohol and tobacco together [8].

The association of tobacco with the risk of cancer may differ among the head and neck cancer subtypes [15]. In some studies, it was demonstrated that smoking had a stronger association with larynx and pharynx than the oral cavity. This may be due to the higher exposure of larynx and pharynx to smoke than the oral cavity [15, 16].

The destructive link between tobacco products and human cancers stems from a powerful combination of two factors - nicotine and carcinogens. Nicotine is addictive and toxic, but there is no scientific evidence that nicotine is carcinogenic, and the IARC does not classify nicotine as a carcinogen. However, this addiction causes people to use tobacco products constantly, and these products contain many carcinogens. Cigarette smoke contains more than 60 carcinogens and unburned tobacco contains at least 16 carcinogens. Among these, tobacco-specific nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN), polycyclic aromatic hydrocarbons (such as benzoapyrene) and aromatic amines (such as 4-aminobiphenyl) seem to play an important role as causes of oral cancer [17].

Some believe that filtering hookah smoke through water reduces nicotine. However, contrary to popular belief, studies have shown that only 5% of nicotine is removed by water. In addition, hookah users may tend to increase the duration of smoking, thereby increasing the concentration of nicotine in their bloodstream. Therefore, considering the aforementioned harmful effects of hookah use and the results of a recent study, it seems that the use of this smoking device may cause changes in the oral mucosa [3].

Studies have shown that tobacco users, including slaked lime in the betel quid or with areca nut, experience carcinogenic and genotoxic effects on human oral epithelial cells. These products produce reactive oxygen species (ROS) in the chewing mouth [18]. Areca nut is composed of phenolic compounds and tobacco releases various nitrosamines in the mouth that are responsible for proliferative abrasions and damage to DNA and fibroblasts [19, 20]. The N-nitroso compound extracted from Areca nuts, which contains the active ingredient 3- (methyl nitrosamino) proprionityl, has been shown to cause gene poisoning and cytotoxicity responsible for tumors in the buccal cavity of smokeless smokers [21].

The long-lasting and frequent presence of paan and gutkha in the mouth around the gums leads to inflammation of the oral mucosa, which causes the activation of T-cells and macrophages, and ultimately the release of prostaglandins.

Prostaglandin production in buccal keratinocytes occurs due to Arka nut extract, which plays an important role in oral tissue fibrosis and cancer. Cytokines such as interferon-α, tumor necrosis factor (TNF), interleukin-6, and growth factor-like transforming growth factor-beta have been found to be produced at the sites of irritation [22]. The nitrosamine in tobacco is metabolized by cytochrome P450 enzymes, which may lead to the formation of N-nitrosonornicotine, a major carcinogen, which can lead to DNA damage and eventually oral cancer [23].

The consumption of tobacco is closely associated not only with the development of oral cancer, but also with the course of disease evolving a poor prognosis. The most widespread form of tobacco is chewing of betel-quid with tobacco and this has been demonstrated as a major risk factor of cancer of oral cavity [24].

Evidence from many studies shows that smoking in any way doubles the risk of oral cancer in men and women. The risk increases significantly with the duration and frequency of smoking. The risk among former smokers is consistently lower than current smokers, and the risk decreases as the years of quitting increase [25].

The highest incidence of tobacco related oral cancer is seen in low and middle income countries. People in the lower socioeconomic strata are more commonly affected. In India almost 21 people per 100 000 of the population are affected [26]. Data from a pioneering study by Taiwanese researchers show that people with a habit of smoking, drinking and chewing betel nuts at the same time are 123 times more likely to develop oral cancer than the general population [27]. More than 50 percent of oral cancers in India, Sudan and the Republic of South Sudan and about four percent of oral cancers in the United States are due to smokeless tobacco products. Smoking smoke-free tobacco is on the rise among young people in South Asia with the marketing of well-packaged products made from areca nuts and tobacco. As a result, oral precancerous conditions are significantly increased in young adults [28, 29].

4.2 Cigarette

Smoking helps to spread the tumor by suppressing immunity and tumor suppressor genes, most importantly p53 and PTEN [5]. The benefit of quitting smoking may be a time-dependent advantage. It was found that the risk of oral cancer among non-smokers is similar to that of former smokers after 10 years of smoking cessation. In addition, quitting smoking later or in middle age may significantly reduce the risk of oral cancer [30, 31].

A study in China, which included 210 cases, reported a strong association between long-term smoking and OSCC [32]. In the study by Ahmed et al. they have reported an increase in nuclear size, nuclearcytoplasmic (N/C) ratio and multi-lobed nuclei, while a decrease in size of cytoplasm in smokers as compared to non smokers [33]. The study of Woyceichoski et al. has also revealed an increase in cytoplasmic size and N/C ratio, while a decrease in size of cytoplasm in cocaine users as compared to the control group [34].

4.3 Alcohol

Tobacco and alcohol are the most well-known reasons for oral and throat cancers [35]. The synergistic effects of alcohol and tobacco smoke increase the risk of OSCC by increasing the permeability of the oral epithelium, tobacco solution, and increasing its permeability [36]. However, chronic alcohol use alone may lead to OSCC through several mechanisms, including the formation of DNA adducts, the production of ethanol-related reactive oxygen species, and interference with the DNA repair mechanism [37].

4.4 Shammah

Shamma consumption is increasing in many countries [38]. It is a combination of smokeless powder tobacco with ingredients such as lime, pepper, ash and flavorings, and people use it by placing it in the buccal cavity until the taste penetrates [39]. Another study of Yemeni shammah users found that there was a strong link between daily consumption of leukoplakia [40].

4.5 Chewing of khat

Khat is a plant that is mostly used for chewing and is amixture of cathine and norephidrine [41]. In an earlier case report of one patient, a strong affiliation

between khat chewing and growth of OSCC was reported [42]. Sawair *et al*. also reported a strong association between khat chewing and OSCC development in their study, which included 649 Yemeni patients [43].

4.6 Shisha (water pipe) smoking

Shisha is commonly available in restaurants, cafes, and other eatery shops in many countries and it contains a high concentration of nicotine, tar, and carbon monoxide [44].

Smoking with water (WPS) is a type of smoking that has traditionally been used for many years in Asia and the eastern Mediterranean. However, in the 1990s, it became global with increasing popularity in other parts of the world, including Western countries [45].

A recent study found a strong link between water pipe smoking and head and neck cancers [45]. In a study from Syria and Lebanon, Zaid et al. Reported that mutations in the p53 gene are smoking in the water tube at OSCC63 [46].

Al-Amad conducted a study in Jordan that found that 36% of those with oral cancer had a habit of smoking in a water pipe [47].

5. Protective factors

Recent studies show that the consumption of coffee, vegetables, fruits, folic acid have a protective effect [8]. A study by Ding et al. In 2013 shows that the use of polyphenols, especially in black and green tea, is effective in preventing oral cancer [48].

6. Disease epidemiology in the world

Oral cancer is a serious and growing problem in many countries. Epidemiological studies show that the incidence of oral cancer and its mortality varies in different parts of the world [49]. In Australia, more than 4,000 new cases of head and neck cancer (including lip cancer) are diagnosed each year. Over 600 of these cancers are oral cavity cancers. In developing countries, oral cancer is the sixth cancer among men and the tenth cancer in women. Worldwide, more than 400,000 new cases of oral cancer are diagnosed each year, two-thirds of which occur in Asian countries such as Sri Lanka, Indonesia, India, Pakistan and Bangladesh [50]. In these high-risk countries, oral cancer is the most common malignancy, accounting for over 25% of all new cases of cancer each year [51]. According to the International Agency for Research on Cancer, some countries in Asia and the Pacific had the top three rates of oral cancer in 2018 [52].

7. Control and prevention measures

Due to recent developments in the diagnosis and treatment of cancers, the survival of patients with cancers of the breast, colon and ovary has increased. However, over the past 50 years, the survival of patients with oral cancer has not changed [53]. In other words, oral cancer has a poor prognosis and the overall 5-year survival rate is 40%, although if diagnosed at an early stage (I and II), the survival rate can exceed 80% [54]. Up to 50% of oral cancers are diagnosed at an advanced stage (stages III and IV) because most patients are asymptomatic in the early stages

and do not seek medical help until they see clear symptoms such as pain, bleeding or a mass in the mouth or neck [55]. When the diagnostic delay is more than a month, the risk of having an advanced stage of oral cancer increases significantly [56]. In most cases, the patient is responsible for a large part of the diagnostic delay. However, delays can also be the result of an incorrect medical approach, as there is no suspicion of oral malignancy and it is not diagnosed and treated in a timely and sufficient manner [56, 57]. Clinical and pathological stage in diagnosis is the most important factor in prognosis [11].

Prevention of this devastating disease can be due to fundamental changes in the socio-economic situation, as well as measures to reduce demand, production, marketing and use of tobacco and alcohol products [58]. A healthy diet, oral hygiene and awareness of the signs and symptoms of the disease are important. Success depends on the political will, intersectoral actions, and culturally sensitive public health messages that are disseminated through educational campaigns and mass media initiatives [59].

Primary prevention of oral cancer therefore consists in education of people on the lifestyle changes such as non-smoking and alcohol consumption and protection from sunlight can reduce the risk of oral cancer [8]. Despite the increasing awareness of oral cancer in the general population, in the last 40 years the percentage of patients seeking medical attention with advanced disease has not changed significantly [51]. At least three-quarters of all oral cancers can be prevented by quitting smoking and drinking alcohol. Eliminating these two known factors also reduces cancer recurrence. In India and Sri Lanka, non-smoking tobacco education programs are designed specifically for adolescents to reduce the incidence of oral cancer. HPV vaccination can also be of importance, even though its effectiveness in not as well defined as it is in the prevention of anogenital and cervical cancer [11].

The goal of secondary prevention is early detection of cancer in the oral cavity in one of accessible places. The chance of curing oral cancers increases if they are diagnosed and treated early. Treatment of early-stage oral cancer increases patient survival. Unfortunately, most oral cancers are diagnosed at a more advanced stage and when they become symptomatic, which greatly reduces a person's chances of recovery, so early detection of precancerous lesions or early-stage oral cancer is very valuable. Diagnosis of suspected cases of oral cancer is made by assessing the patient's demographic characteristics and assessing specific habits, especially tobacco and alcohol consumption and other irritating factors that may play a role in causing oral cancer.

Routine biopsy in people with clinically characteristic precancerous lesions may lead to early detection of the underlying cause of oral cancer. In addition to history, physical examination, and biopsy, simultaneous evaluation of the upper aerodigestive tract is essential because patients with oral cancer are at risk for cancer of other parts of the head, neck, and lungs [25].

Oral health status and family history should also be evaluated for any syndromes that may increase the risk of oral cancer. In addition to the history, a complete examination of the head and neck is performed to carefully examine the location and spread of the primary tumor and identify metastases. It is noteworthy that early-stage cancerous lesions may be red or white plaques and non-ulcerative. More advanced cancers are ulcerative, aggressive, fungal, and prominent, or both. Cancer may develop within precancerous lesions such as leukoplakia or erythroplakia. Therefore, increasing the awareness of dentists is very important in getting a complete history and examination of the head and neck. Symptoms to consider include:

- Swelling or lumps on the lips or inside the mouth
- Wounds on the face, neck or mouth that do not heal within 2 weeks.

- Wounds under the denture
- White, red lesions
- Bleeding in the mouth for no reason
- Numbness or tenderness and unexplained pain in any area of the face, mouth and neck
- Difficulty swallowing, chewing, talking or moving the jaw or tongue
- Hoarseness, persistent sore throat or voice change
- Weight Loss [8].

Unlike other frequent cancers (for example, colon or cervical cancer), a standard population-based screening program for oral cancer is not cost-effective and cannot be recommended [51]. Screening programs can be valuable in patients from high-risk groups (smokers and alcoholics) or in patients with a previous diagnosis of cancer outside the head and neck [60]. In countries with regular dental practice attendance, opportunistic screening for oral mucosal lesions (early-stage cancer or precancerous lesions) in general dental practice could also be relevant in reducing diagnostic delay [61].

Visual screening involves regular visual and physical examination of the intraoral mucosa under intense light to observe the symptoms of oral potentially malignant disorders (OPMD) as well as early oral cancer, followed by careful examination and digital palpation of the neck for lymph node enlargement. This is a provider-dependent mental test. Accordingly, its performance in detecting lesions varies among providers. Comprehensive knowledge of oral anatomy, natural history of oral carcinogenesis, and clinical-pathological features of OPMDs and preclinical cancers are important prerequisites for effective oral vision screening providers [59]. A significant 34% reduction in oral cancer mortality among a high-risk group of smokers and alcoholics after three rounds of oral vision screening has been shown in a randomized controlled cluster trial in India [62, 63]. A 15-year follow-up showed a steady decline in oral cancer mortality, with a further decline in those who adhere to frequent screening courses. 38% reduction in oral cancer incidence (95% CI 8–59%) and 81% reduction in oral cancer mortality (95% CI 69–89%) in tobacco and /or alcohol users who They were screened four times [62].

Known risk factors, long natural history, easy diagnosis of precancerous lesions by oral examination make oral cavity cancer very suitable for population screening. Oral cancer usually occurs in accessible places, which can be diagnosed early by visual inspection and touch. Therefore, oral self-examination is possible for everyone because it is a method for early detection of precancerous oral lesions without the need for a simple, non-invasive and inexpensive healthcare professional [64]. It should be strongly supported for ordinary people, especially high-risk people [52]. A quasi-experimental study in Australia found the importance of oral self-examination in reducing the incidence and mortality of oral cancers [65].

Also, prompt treatment is essential for successful secondary prevention. Secondary prevention is also called cancer control [66]. Surgery and radiation therapy are widely used to treat premature oral cancer, either alone or in combination. The choice of method depends on the location of the tumor, cosmetic and functional outcomes, patient age, comorbidities, patient preference, and specialization [59].

The third prevention targets the final stages. More than 70% of advanced cancers have severe pain and other distressing symptoms. Pain control and palliative care are the third most important prevention strategies [67].

8. Conclusion

Since oral cancer is the sixth most common cancer in men and the tenth most common cancer in women and puts a lot of burden on health care providers and the public, and on the other hand many of its risk factors such as smoking and hookah can be controlled, including screening and diagnosis Early in the health care system and educational intervention programs are recommended.

Conflict of interest

The authors declare no conflict of interest.

Abbreviation

PMOEL	Potentially malignant oral epithelial lesions
OSCC	oral squamous cell carcinoma
OPMD	observe the symptoms of potentially malignant oral disorders

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References

[1] Zinat Motlagh SF, Chaman R, Ghafari SR, Parisay Z, Golabi MR, Eslami AA, et al. Knowledge, treatment, control, and risk factors for hypertension among adults in Southern Iran. International journal of hypertension. 2015;2015.

[2] GHAJARIEH SS, Kamangar F, Poustchi H, Malekzadeh R. Reducing the burden of chronic diseases: a neglected agenda in Iranian health care system, requiring a plan for action. 2010.

[3] Taghibakhsh M, Farhadi S, Babaee A, Sheikhi M. The effect of hookah use on buccal mucosa: Evaluation of repair index. Asian Pacific journal of cancer prevention: APJCP. 2019;20(4):1109.

[4] Jeihooni AK, Harsini PA. The Effect of an Educational Intervention Based on PRECEDE Model on Oral Cancer Prevention Behaviors in Hookah Users. Journal of Cancer Education. 2020; 35(6):1250-60.

[5] Bugshan A, Farooq I. Oral squamous cell carcinoma: metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Research. 2020;9.

[6] Joshi P, Dutta S, Chaturvedi P, Nair S. Head and neck cancers in developing countries. Rambam Maimonides medical journal. 2014;5(2).

[7] Wong T, Wiesenfeld D. Oral cancer. Australian dental journal. 2018;63: S91-S9.

[8] Contributing authors supervised by Dr. Parvin Yavari. Epidemiology textbook of prevalent diseases in Iran (cancers). first edition. volume 3. Tehran. 2014.

[9] Rivera C. Essentials of oral cancer. International journal of clinical and experimental pathology. 2015;8(9): 11884.

[10] Villa A, Villa C, Abati S. Oral cancer and oral erythroplakia: an update and implication for clinicians. Australian dental journal. 2011;56(3):253-6.

[11] Chow LQ. Head and neck cancer. New England Journal of Medicine. 2020;382(1):60-72.

[12] Rastam S, Ward KD, Eissenberg T, Maziak W. Estimating the beginning of the waterpipe epidemic in Syria. BMC public health. 2004;4(1):1-5.

[13] Khalil J, Heath RL, Nakkash RT, Afifi RA. The tobacco health nexus? Health messages in narghile advertisements. BMJ Publishing Group Ltd; 2009.

[14] Kuper H, Adami HO, Boffetta P. Tobacco use, cancer causation and public health impact. Journal of internal medicine. 2002;251(6):455-66.

[15] Dhull AK, Atri R, Dhankhar R, Chauhan AK, Kaushal *V. Major* risk factors in head and neck Cancer: a retrospective analysis of 12-year experiences. World journal of oncology. 2018;9(3):80.

[16] Maasland DH, van den Brandt PA, Kremer B, Goldbohm RAS, Schouten LJ. Alcohol consumption, cigarette smoking and the risk of subtypes of head-neck cancer: results from the Netherlands Cohort Study. BMC cancer. 2014;14(1):1-14.

[17] Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature Reviews Cancer. 2003;3(10):733-44.

[18] Babu S, Sesikeran B, Bhat R. Oral fibrosis among teenagers chewing tobacco, areca nut, and pan masala. The Lancet. 1996;348(9028):692.

[19] Nair J, Ohshima H, Friesen M, Croisy A, Bhide SV, Bartsch H. Tobacco-specific and betel nut-specific N-nitroso compounds: occurrence in saliva and urine of betel quid chewers and formation in vitro by nitrosation of betel quid. Carcinogenesis. 1985;6(2): 295-303.

[20] Bhide S, Gothoskar S, Shivapurkar N. Arecoline tumorigenicity in Swiss strain mice on normal and vitamin B deficient diet. Journal of cancer research and clinical oncology. 1984;107(3):169-71.

[21] Chiu C-J, Chang M-L, Chiang C-P, Hahn L-J, Hsieh L-L, Chen C-J. Interaction of collagen-related genes and susceptibility to betel quid-induced oral submucous fibrosis. Cancer Epidemiology and Prevention Biomarkers. 2002;11(7):646-53.

[22] Kandasamy M, Anisa N, Rahman A, Rajan MA, Prakash A, Lal J. Etiopathogenesis of oral submucous fibrosis-review of literature. Journal of Advanced Medical and Dental Sciences Research. 2015;3(3):53.

[23] Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. Mutagenesis. 2004;19(4):251-62.

[24] Mathur A, Jain M, Shiva M, Navlakha M, Kulkarni S. Tobacco habits and risk of oral cancer: A retrospective study in India. Iranian Journal of Blood and Cancer. 2009;1(3):111-6.

[25] Sankaranarayanan R, Ramadas K, Amarasinghe H, Subramanian S, Johnson N. Oral cancer: prevention, early detection, and treatment. 2015.

[26] Chaturvedi P, Singh A, Chien C-Y, Warnakulasuriya S. Tobacco related oral cancer. BMJ. 2019;365.

[27] Guo S-E, Huang T-J, Huang J-C, Lin M-S, Hong R-M, Chang C-H, et al. Alcohol, betel-nut and cigarette consumption are negatively associated with health promoting behaviors in Taiwan: a cross-sectional study. BMC public health. 2013;13(1):1-8.

[28] Sinha D, Palipudi K, Rolle I, Asma S, Rinchen S. Tobacco use among youth and adults in member countries of South-East Asia region: review of findings from surveys under the Global Tobacco Surveillance System. Indian journal of public health. 2011;55(3): 169-76.

[29] Gupta B, Ariyawardana A, Johnson NW. Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives. International dental journal. 2013;63(1): 12-25.

[30] Porter S, Gueiros LA, Leão JC, Fedele S. Risk factors and etiopathogenesis of potentially premalignant oral epithelial lesions. Oral surgery, oral medicine, oral pathology and oral radiology. 2018;125(6):603-11.

[31] Warnakulasuriya S, Dietrich T, Bornstein MM, Peidró EC, Preshaw PM, Walter C, et al. Oral health risks of tobacco use and effects of cessation. International dental journal. 2010;60(1):7-30.

[32] Wang X, Xu J, Wang L, Liu C, Wang H. The role of cigarette smoking and alcohol consumption in the differentiation of oral squamous cell carcinoma for the males in China. Journal of cancer research and therapeutics. 2015;11(1):141.

[33] Ahmad H, Ebnoof S. Oral epithelial atypical changes in apparently healthy oral mucosa exposed oral mucosa to smoking, alcohl, peppers and hot meals, using the AgNOR and papanicolaou staining techniques. 2009.

[34] Woyceichoski IEC, de Arruda EP, Resende LG, Machado MAN, Grégio AMT, Azevedo LR, et al. Cytomorphometric analysis of crack cocaine effects on the oral mucosa. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2008;105(6):745-9.

[35] Fu YS, Wenig BM, Abemayor E. Head and neck pathology with clinical correlations: Churchill Livingstone; 2001.

[36] Feller L, Chandran R, Khammissa R, Meyerov R, Lemmer J. Alcohol and oral squamous cell carcinoma: clinical review. South African Dental Journal. 2013;68(4): 176-80.

[37] Liu Y, Chen H, Sun Z, Chen X. Molecular mechanisms of ethanol-associated oro-esophageal squamous cell carcinoma. Cancer letters. 2015;361(2):164-73.

[38] Alharbi F. Incidence of head and neck cancers in Jazan province, Saudi Arabia. Saudi Journal of Otorhinolaryngology Head and Neck Surgery. 2017;19(2):47.

[39] Quadri MFA, Alharbi F, Bajonaid AMS, Moafa IHY, Sharwani AA, Alamir AHA. Oral squamous cell carcinoma and associated risk factors in Jazan, Saudi Arabia: a hospital based case control study. Asian Pacific Journal of Cancer Prevention. 2015;16(10):4335-8.

[40] Scheifele C, Nassar A, Reichart P. Prevalence of oral cancer and potentially malignant lesions among shammah users in Yemen. Oral oncology. 2007;43(1):42-50.

[41] Al-Hebshi N, Skaug N. Khat (*Catha edulis*)—an updated review. Addiction biology. 2005;10(4):299-307.

[42] Fasanmade A, Kwok E, Newman L. Oral squamous cell carcinoma associated with khat chewing. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2007;104(1):e53-e5.

[43] Sawair FA, Al-Mutwakel A, Al-Eryani K, Al-Surhy A, Maruyama S, Cheng J, et al. High relative frequency of oral squamous cell carcinoma in Yemen: qat and tobacco chewing as its aetiological background. International journal of environmental health research. 2007;17(3):185-95.

[44] Shafagoj YA, Mohammed FI. Levels of maximum end-expiratory carbon monoxide and certain cardiovascular parameters following hubble-bubble smoking. Saudi medical journal. 2002;23(8):953-8.

[45] Patil S, Awan KH, Arakeri G, Aljabab A, Ferrari M, Gomes CC, et al. The relationship of "shisha"(water pipe) smoking to the risk of head and neck cancer. Journal of Oral Pathology & Medicine. 2019;48(4):278-83.

[46] Zaid K, Azar-Maalouf E, Barakat C, Chantiri M. p53 overexpression in oral mucosa in relation to Shisha smoking in Syria and Lebanon. Asian Pacific journal of cancer prevention: APJCP. 2018;19(7):1879.

[47] Al-Amad SH, Awad MA, Nimri O. Oral cancer in young Jordanians: potential association with frequency of narghile smoking. Oral surgery, oral medicine, oral pathology and oral radiology. 2014;118(5):560-5.

[48] Ding Y, Yao H, Yao Y, Fai LY, Zhang Z. Protection of dietary polyphenols against oral cancer. Nutrients. 2013;5(6):2173-91.

[49] Jeihooni AK, Dindarloo SF, Harsini PA. Effectiveness of health belief model on oral cancer prevention in smoker men. Journal of Cancer Education. 2019;34(5):920-7.

[50] Montero PH, Patel SG. Cancer of the oral cavity. Surgical Oncology Clinics. 2015;24(3):491-508.

[51] Abati S, Bramati C, Bondi S, Lissoni A, Trimarchi M. Oral Cancer and Precancer: A Narrative Review on the Relevance of Early Diagnosis. International Journal of Environmental Research and Public Health. 2020;17(24):9160.

[52] Hung L-C, Kung P-T, Lung C-H, Tsai M-H, Liu S-A, Chiu L-T, et al. Assessment of the risk of oral cancer incidence in a high-risk population and establishment of a predictive model for oral cancer incidence using a population-based cohort in Taiwan. International journal of environmental research and public health. 2020;17(2):665.

[53] Wong DT. Towards a simple, saliva-based test for the detection of oral cancer. 'Oral fluid (saliva), which is the mirror of the body, is a perfect medium to be explored for health and disease surveillance.'. Expert review of molecular diagnostics. 2006;6(3):267-72.

[54] Silverman S, Kerr AR, Epstein JB. Oral and pharyngeal cancer control and early detection. Journal of Cancer Education. 2010;25(3):279-81.

[55] McCullough M, Prasad G, Farah C. Oral mucosal malignancy and potentially malignant lesions: an update on the epidemiology, risk factors, diagnosis and management. Australian dental journal. 2010;55:61-5.

[56] Gómez I, Seoane J, Varela-Centelles P, Diz P, Takkouche B. Is diagnostic delay related to advanced-stage oral cancer? A meta-analysis. European journal of oral sciences. 2009;117(5):541-6.

[57] Groome PA, Rohland SL, Hall SF, Irish J, Mackillop WJ, O'Sullivan B. A population-based study of factors associated with early versus late stage oral cavity cancer diagnoses. Oral oncology. 2011;47(7):642-7.

[58] Johnson NW, Warnakulasuriya S, Gupta P, Dimba E, Chindia M, Otoh E, et al. Global oral health inequalities in incidence and outcomes for oral cancer: causes and solutions. Advances in dental research. 2011;23(2):237-46.

[59] Sankaranarayanan R, Ramadas K, Amarasinghe H, Subramanian S, Johnson N. Oral cancer: prevention, early detection, and treatment. Cancer: disease control priorities, third edition (volume 3). 2015.

[60] Van der Waal I, de Bree R, Brakenhoff R, Coebegh J. Early diagnosis in primary oral cancer: is it possible? Medicina oral, patologia oral y cirugia bucal. 2011;16(3):e300-e5.

[61] Lim K, Moles D, Downer M, Speight P. Opportunistic screening for oral cancer and precancer in general dental practice: results of a demonstration study. British dental journal. 2003;194(9):497-502.

[62] Sankaranarayanan R, Ramadas K, Thara S, Muwonge R, Thomas G, Anju G, et al. Long term effect of visual screening on oral cancer incidence and mortality in a randomized trial in Kerala, India. Oral oncology. 2013;49(4):314-21.

[63] Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, et al. Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. The Lancet. 2005;365(9475):1927-33.

[64] Shrestha G, Maharjan L. Mouth Self-Examination for Prevention and Control of Oral Cavity Cancer. JNMA; journal of the Nepal Medical Association. 2020;58(225):360-2.

[65] Jornet PL, Garcia FG, Berdugo ML, Perez FP, Lopez APF. Mouth self-examination in a population at risk of oral cancer. Australian dental journal. 2015;60(1):59-64.

[66] Alonge O, Narendran S. Opinions about oral cancer prevention and early detection among dentists practising along the Texas–Mexico border. Oral diseases. 2003;9(1):41-5.

[67] Mangalath U, Aslam SA, Khadar AHKA, Francis PG, Mikacha MSK, Kalathingal JH. Recent trends in prevention of oral cancer. Journal of International Society of Preventive & Community Dentistry. 2014;4(Suppl 3):S131.

Chapter 2

Diagnostic Potential of Salivary Exosomes in Oral Cancer

Henry Ademola Adeola, Haly Holmes and Dada Oluwaseyi Temilola

Abstract

"Omics" based concepts and techniques are gaining momentum in the field of oral medicine, spurred on by rapid advancements within the field of precision diagnostics and therapeutics. Oral cancer, specifically oral squamous cell carcinoma is the most common head and neck cancer, posing both diagnostic and prognostic challenges globally. Saliva offers several advantages as a diagnostic tool and has gained recognition as a biological medium for liquid biopsy. Salivary biomarkers, such as exosomes not only contain the full spectrum of genomic, lipidomic and proteomic material from its cell of origin, but are also more stable and consistently measurable in saliva due to their phospholipid structural protection of their merchandise/contents. Salivary exosomes are mediators in communication and transfer of contents between cancer and normal cells and thus key role players in mediating the tumor environment. Even though exosomes have been widely employed to investigate systemic diseases including head and neck cancers, unraveling the biologic mechanisms, scope of application of salivary tumor-derived exosomes and overcoming restrictions in this emergent field of saliva-exosomics warrants further investigation.

Keywords: Saliva, exosomes, oral cancer, exosomics, omics-based approaches

1. Introduction

Despite the fact, that there is a relatively wider coverage and application of several emerging molecular and omics-based techniques, in the field of medicine; many of these concepts (and techniques) are only beginning to gain recognition in the field of dentistry [1]. The completion of the human genome project [2–4], has expanded the trajectory for precision diagnostic and therapeutic potential thereof across many biomedical fields [5, 6]. The clinical lexicon in the post-genomic era is now burgeoning with various personalized application of genomics (and phenomics) to improving the cancer management pipelines [1, 6–9].

Orthogonal (but complementary) multimodal approaches to conventional histopathology [10, 11], such as liquid biopsies technologies have emerged as useful tools for clinical oncology [11–13], early tumor diagnosis and biomonitoring [14–17], as well as therapeutic decision making and delivery [18]. Nano-scaled multivesicular exosomes have emerged as important components of the tumor circulome that has significantly improved the cancer diagnostic field [11, 14]. Furthermore, salivary exosomes have been applied to improve the diagnosis of various cancers [19–26],

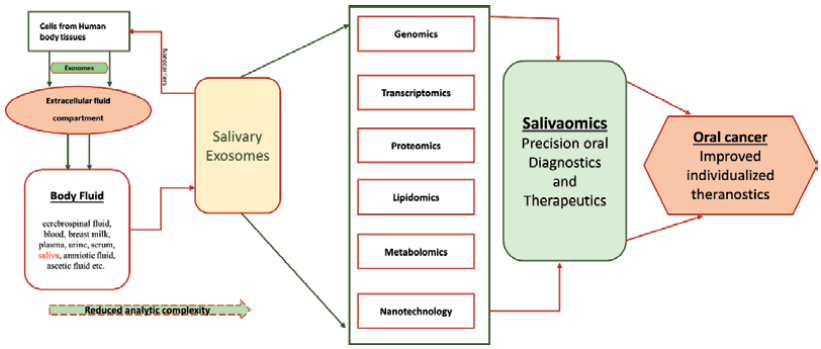

Figure 1.
Overview of origin and theranostic value of exosomes in oral cancer.

including oral cancers [25, 26]. The focus of this chapter is to review the applications and prospects of salivary exosomes in oral cancer detection (**Figure 1**).

2. Salivary diagnostics

Human saliva is a multifunctional biological fluid, which facilitates digestion, swallowing, tasting, tissue lubrication and protection against infectious organisms. It is comprised largely of water (99%) and other biochemical substances such as proteins, nucleic acids, mucins, immunoglobulins, a variety of electrolytes and lipids [27, 28]. Whole saliva production is derived from all the salivary glands including the gingival crevicular fluid [29]. Saliva, which has been used for diagnostic purposes for over 2000 years, plays a key role in the maintenance of general and particularly oral health homeostasis [30]. The health of individuals has been determined by salivary changes such as amount produced, smell, ropiness and gustatory sensation [31, 32].

Components of the salivary glands are responsible for production, modification, transportation and secretion of saliva into the oral cavity (via acinar cells, various ductal system cells, and myoepithelial cells) [33]. The close proximity of a network of highly permeable blood capillaries to the saliva producing acinar cells facilitates the free exchange of blood-derived molecules into the acinar cells [34], which enter the salivary tissues either via transcellular (passive and active transport) or paracellular (extracellular ultrafiltration) routes [35, 36]. This transfer potentially influences the molecular constituency of oral fluids. Diffusion is the most common transport mechanism of molecules from blood into the saliva and this process is driven/influenced by the size and the electric charge of the molecules [37]. Active transport involves the transcellular transportation of blood into saliva via secretory acinar cells of the salivary glands. Ultra-filtration is the major mode by which molecules are transported into saliva via the paracellular route, whereby small sized blood molecules filter into saliva through the spaces between ductal and acinar cells [37]. Salivary acini secrete saliva into collecting ducts, where sodium reabsorption, and bicarbonate and potassium are secretion takes place, thus altering the composition of the saliva [38, 39]. Even though blood molecules transported through ultra-filtration into saliva are usually in low concentration in comparison to their levels in blood, this mode of transport enables blood molecules such as DNA, RNA, proteins, metabolites and exosomes into saliva through the salivary gland. This confers the possibility for oral fluids to harbor molecular information indicative of an individual's current state of health. This information may be reflected by changes in the concentrations of these molecules or mutation in the genetic constitution of the

molecules which are also present in saliva - serving as potential salivary biomarkers for diagnosis, prognosis and monitoring of therapeutic responses [40].

Many body fluids have been explored as alternative sources for biomarkers in molecular diagnosis of cancers, genetic, immunological and other systemic diseases [41–45]. However, blood, urine and saliva are the most used media for discovery of biomarkers [46]. Saliva is a rich source of proteins and its DNA, RNA, and protein content is analogous to that of blood with significant commonality in hormones, antibodies and other molecules. Most of the resident salivary protein constituents are synthesized within the salivary glands, with the rest transported from blood or lymph into saliva and used as biomarkers for disease diagnosis and screening purposes [47].

Saliva has the advantages over blood in that it is a readily available specimen which can be collected by non-invasive techniques and is recommended as the diagnostic medium in vulnerable populations such as children. The retrieval of multiple salivary samples from the same individual is possible with minimal discomfort and saliva is safer to work with when compared with blood samples. For example, there are some factors in saliva which help to inhibit HIV infectivity thereby limiting the rate of HIV transmission through the oral cavity [48]. Saliva sample processing is more economical and does not clot making it easier to store and ship with less manipulation. However, the low level of protein detection in saliva is sensitive to the method of saliva collection and specimen contamination. Normal high-abundance salivary constituents such as amylase and proline rich proteins (during stimulated salivary collection methods especially), may dilute the presence of low-level proteins, which may be more important biomarkers.

Salivary biomarkers are miniscule and measured in (picograms), detection of which can only be achieved by techniques which are both sensitive and specific enough to discriminate between them [49]. Technological advances in diagnostic detection methods (next generation sequencing, mass spectrometry, genome wide association studies and other screening techniques) have paralleled the demand for improved diagnostic test accuracy of salivary genomic and proteomic biomarkers, thereby conferring distinct advantages for saliva in the diagnosis and monitoring of diseases such as oral cancer and precancer.

Salivary exosomes, which are nano-sized salivary biomarkers equipped with all the molecular cargo from the parent cells, have become increasingly detectable due to their stability in the circulation and bodily fluids. They have been extensively explored as diagnostic tools for local and systemic diseases [50].

2.1 Salivary exosome physiology

Fusion of nano-sized (30–100 nm in diameter) multivesicular bodies (MVB) [51] derived from the endocytic pathway with plasma membrane was discovered over 30 years ago [52]. Johnstone et al., detected the release of small vesicles into extracellular spaces by reticulocyte MVB's and observed that their enzymatic activity mirrored that of the cell culture from which they were shed [52]. These bodies (exosomes), originally believed to be involved in waste disposal, due to their resistance to degradation by lysozymes, is now more understood to subserve vital biological functions when released as extracellular vesicles [53]. These functions are influenced/determined by their target cell with which they interact and include cellular communication and homeostasis, immune control (they contain IgA), RNA processing and transport of drugs [53–55].

Exosomal release (after formation of intraluminal vesicles), can be compared to the reversal of the endocytosis process, permits their evaluation in the extracellular body fluid environments [56]. The exocytotic release of exosomes into the extracellular domain, reveals that they naturally contain key molecular components derived

from the parent cell relating to membrane transport, lipid metabolism and extracellular matrix formation [21, 57]. In addition, cytoplasmic nucleic acid contents such as mRNA and microRNA have been found in exosomes [21, 58, 59]. Considering the important contents as highlighted here, exosomes have been identified to play crucial roles in cell-to-cell communication [21]. All exosomes regardless of their origin possess both shared conserved and cell-specific proteins. Emerging knowledge has associated exosomes with the development of physiological and pathological perturbations [60, 61]. For instance, cancer exosomes have been found to be capable of a range of tumor-promoting activities, such as immunomodulation, development of pre-metastatic niches, as well as dysregulation of angiogenesis [62–64]. Furthermore, cancer exosomes are vital indicators of potentially malignant events in the tumor microenvironment and may exhibit pheno-genomic perturbation biomarkers of cancer [65, 66].

2.2 Diagnostic benefits of exosomes in body fluids

Due to exosomal release into the extracellular compartment, they are abundantly found in most body fluids such as cerebrospinal fluids, blood, breast milk, plasma, urine, serum, saliva, amniotic fluid, semen and ascetic fluid [21, 52–55]. Exosomes are highly suitable substrates for biomarker signature discovery. Because of the content-protective packaging of their rich cargo (by lipid membranes) from extracellular lytic enzymes, and significantly lower complexity of its contents in comparison to whole tissue analysis [53].

Analyzing exosomal shuttle RNA (esRNA) in maternal blood, has been proposed as a potential surrogate prenatal diagnostic tool, to avoid risky invasive procedures such as chorionic villus sampling and amniocentesis [54]. This could potentially lower the risk of surgical injuries and miscarriages. Even though, cell free fetal DNA (cffDNA) has been previously used for the prenatal diagnostic purposes, the low content of fetal cffDNA has reduced the accuracy of this method [54, 56]. Information about cancer risk and genetic disease predisposition can be potentially gleaned from exosomal esRNA content analysis.

The use of novel liquid biopsy-based cancer diagnostic tools has significantly improved the precision and efficacy of individualized medicine, particularly in resource-limited settings [11]. Exosomes are capable of providing robust molecular tumor information about cells of origin, are retrievable from easily accessible body fluids; and hence are highly useful for early detection and follow-up of cancer [55].

2.3 Salivary exosomes and oral cancer

Exosomes have been successfully isolated from saliva [19–25]; and the presence of lipids, proteins and nucleic acids in exosomes, makes salivary exosomes attractive substrates for omics analysis (a.k.a Salivaomics) [57–60]. It has become emergent, that salivary constituents (e.g. mRNAs, proteins, miRNAs, microbes and metabolites such as lipids) may be detected in exosomes and be used as biomarkers for diseases (both local and distal) [54]. Structural, proteomics and transcriptomics analysis of salivary exosomes has been successfully carried out [61–65]; and salivary exosomes are fast becoming key tools in cancer biomarker theranostics. Exosomes have been revealed as valuable indicators of the micro-environments and perpetrators of cancer intercellular communication [25].

The mean diameter and protein content are used as the basis upon which exosomes isolated from saliva are structurally subdivided into two types (I and II which are ca. 85 nm and 40 nm in diameter, respectively) [20]. Since epithelial barriers between blood vessels and salivary gland structures can be crossed by exosomes [66, 67], they have become important tools for essential transport of key signatures

between blood and saliva (which is believed to be an ultrafiltrate of blood) [67–69]. Important molecular information may be exchanged via transudation, ultrafiltration or selective transport based on their size or presence of transporter molecules [67].

Of all the head and neck cancers, oral squamous cell carcinoma is the most prevalent, often diagnosed in advanced stage and is associated with a low survival and poor prognosis. Early detection of oral cancer is a key goal in epidemiological cancer control and successful management thereof. Using salivary exosomes for identification of oral cancer biomarkers is potentially a highly sensitive, cost-effective and non-invasive point-of-care technique for detecting oral cancer that may be subclinical or missed by routine histological diagnostic approaches [70]. Evaluation of these vesicles shed by cancer cells via multivesicular bodies (MVB's) into saliva is a viable approach for biomarker detection in oral cancer (**Figure 2**). Salivary exosomes have played key roles in diagnosis of systemic diseases [51] and have been key player in the molecular characterization of cancer [24]. Due to its reduced complexity as compared to other body fluids, exosomes are reliable tools for early diagnosis of oral cancer and its use as a diagnostic tool may significantly improve cancer survival rates [24].

Due to its nano-scaled structure, human salivary exosomes have been identified as potential carriers for non-invasive delivery of cancer biomarkers [22]. For example, electrochemical sensing methods, such as electric field-induced release and measurement (EFIRM) approach, has been used to improve the field of salivary liquid biopsy [23]. Not least, salivary exosomes have been used to enhance the detection of human papilloma virus (HPV) positive oropharyngeal cancers [26].

The critical role of tumor-derived exosome in cancer in largely due to the presence of tumor-specific signatures within its functional cargos, which includes proteins, miRNA and mRNA (**Table 1**).

The significant physiological interaction and overlap of the blood and salivary proteome (ca. 20–30%) makes exosomal protein biomarkers attractive and many cancer-related exosomal proteins have been identified from oral cancer [23, 82, 83]. Potential proteomic biomarkers of oral cancer such as CFB, CD59, A1BG, M2b, CAT, MRP14, PFN, M2BP, ADA, S100, CFL1, IGHG, TF, IL-1B and IL-8S have been

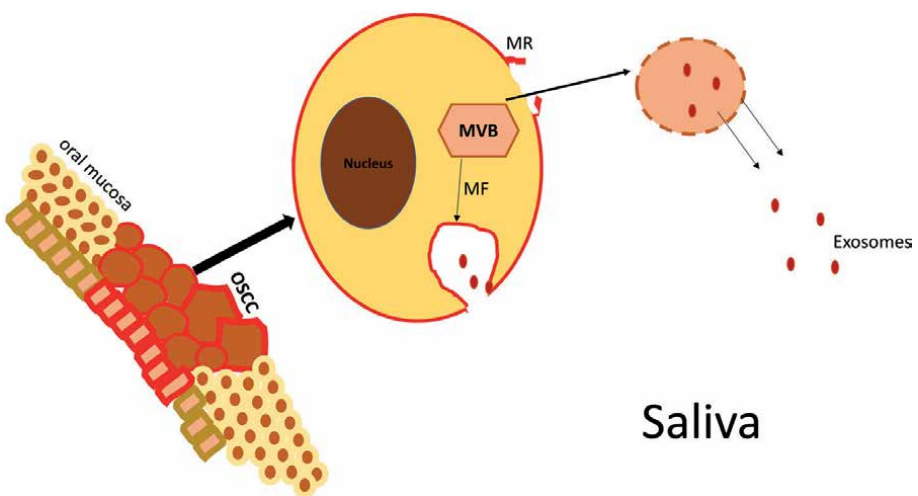

Figure 2.
Pathways for escape of exosomes into saliva. Oral squamous carcinoma cells (OSCC) may release exosomes into saliva, either by fusion of the multivesicular body (MVB) with the plasma membrane (MF) or by plasma membrane rupture (MR) and direct release through endosomal membrane.

Biomarker	Type	Sample	Methods	References
A2M, HPA, MUC5B, LGALS3BP, IGHA1, PIP, PKM1/ M2, GAPDH	Protein	saliva	Mass spectrometry analysis and proteomics data analysis	Winck et al. [71]
miRNA-21	miRNA	OSCC cell line	miRNA sequencing	Li et al. [72]
miR-1246	miRNA	OSCC cell line	MicroRNA microarray	Sakha et al. [73]
miR-200c-3p	miRNA	OSCC cell line	integrated microarray	Kawakubo-Yasukochi et al. [74]
miR-34a-5p	miRNA	OSCC cell line	miRNA sequencing	Li et al. [75]
PF4V1, CXCL7, F13A1, and ApoA1	protein	serum	RT-PCR	Li et al. [76]
miR-101-3p	miRNA	OSCC cell line	Microarray analysis	Xie et al. [77]
miR-382-5p	miRNA	OSCC cell line	RT-PCR	Sun et al. [78]
miR-24-3p	miRNA	saliva	RT-PCR	He et al. [79]
miR-21-5p	miRNA	OSCC cell line	RT-PCR	Chen et al. [80]
miR-155	miRNA	OSCC cell line	RT-PCR.	Kirave et al. [81]

Table 1.
Exosome biomarker for oral cancers.

identified from whole saliva [84–88]. However, protein biomarkers such as MUC5B, A2M, LGALS3BP, HPa, GAPDH, IGHA1, PIP and PKM1/M2 have been specifically identified to be exosomal protein biomarkers of oral cancer with a classification accuracy of 90% [80, 89]. Zlotogorski-Hurvitz et al. (2016), identified CD9/−81 downregulation and CD 63 upregulation in exosomes as early diagnostic protein biomarkers of oral cancer [90]. Furthermore, salivary exosomes isolated from HPV-positive oropharyngeal cancer cell lines has been found to underexpress cyclin D1 and p53 and overexpress p16, T-cell inhibitory protein PTPN11 and E6/E7 proteins [91].

Via their interaction with mRNA, micro RNA's (miRNA) are involved in a number of physiological and disease processes (when there is aberrant expression). MiRNAs are small non-coding RNAs that mediate destabilization and/or translational repression of target messenger RNA (mRNA) molecules thereby reducing final protein output. Exosomes are a rich source of miRNA's, provides a vehicle for cell to cell transporting to alter cellular functions as well as offer protection in the extracellular environment. Exosomal miRNAs have been investigated as candidate screening tools (miR-24-3p) [92], in chemoresistance (miR-21) [81], regulating tumor progression (miR-34-5p) [93] and miR-342–3p and miR-1246 [93] and miR-382-5p [78].

In oral cancer, the intercellular transfer of molecules (such as miRNA's) by cancer associated fibroblast influences the tumor microenvironment. miR-21 represents one of the most abundant miRs transported within EV cargos secreted by oral cancer cells and is a well-established oncogenic miR whose major targets include the tumor suppressors. Its exosomal hideout contributes towards chemoresistance

due to the camouflage provided by its vehicle during intercellular transfer of the oncogenic miR. It is thus also an important chemotherapeutic precision target in cancers [81]. Li et al. [94], in a study of 108 patients with OSCC, observed that tumor exosomal miR 21 was upregulated in hypoxic cancer cells as well as internalized by normoxic cells. Exosomal miR-34-5p transfer between CAF's and neighboring OSCC cells played an important role in regulating tumor progression. Sakha et al. [73], demonstrated that effect that intercellular transfer of exosomal oncogenic miRNA's (miR-342–3p and miR-1246) could be delivered were evaluated for their role in could have on cancer development and progression by influencing cell motility and invasiveness [73]. Exosomal miR-382-5p in cancer-associated fibroblast (CAF) mediated OSSC migration and invasion by evaluation of tissue samples from 47 patients who had OSSC tumor resection. The results showed that CAF's transfer miR-382-5p associated with migration and invasion [78]. The expression of exosomal miR-24-3p was found to be higher in salivary exosomes from OSCC patients compared to healthy controls. The AUC for miR-24-3p was 0.738 and could significantly distinguish OSCC patients from normal individuals with 64.4% sensitivity and 80% specificity in 49 patients with OSSC.

The functional cargos which include mRNA has been considered as potential biomarker in the diagnosis and monitoring and treatment of cancer. Valadi et al. first described the presence of mRNAs in exosomes in 2007 [95]. Subsequently, studies have shown the transfer of bioactive mRNAs (tumor suppressor genes or oncogenes) from a malignant cell to a normal cell led to a change in the phenotype and malignant transformation of the normal cell [95–97].

Few studies have identified mRNA in saliva of oral cancer patients. Li et al. identified potential mRNA biomarker which include IL8, SAT, DUSP, IL1B, OAZ1, H3F3A and S100P, in saliva from oral cancer patients [98]. The study showed that the combination of these biomarkers was highly sensitive and specific in differentiating between OSSC and healthy [98]. An in vitro study showed that oral squamous cell carcinoma cell line (PCI-13, UMSCC47) triggered significant increase expression level of IGFBP-3 mRNA and VEGF mRNA in recipient cells [99].

Even though exosomes have been widely employed to investigate head and neck squamous cell carcinomas [100–103], unraveling the biologic mechanisms and application of salivary tumor -derived exosomes is still an evolving science [67]. This emergent field of saliva-exosomics warrants further investigation.

3. Conclusions

Salivary exosomes provide viable, consistent and stable sources of cancer biomarkers. The scope of its utility as well as understanding the molecular mechanisms which underpins it, requires further investigation. Future studies refining the methodology for extracellular vesicle isolation and cleansing presents the greatest challenge that is needed to overcome the restrictions to exploring the full scope of salivary exosomes in systemic diseases including oral cancer.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Adeola HA, Soyele OO, Adefuye AO, Jimoh SA, Butali A. Omics-based molecular techniques in oral pathology centered cancer: Prospect and challenges in Africa. Cancer Cell International. 2017;**17**:61

[2] Olson MV. The human genome project. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**:4338-4344

[3] Collins FS, Morgan M, Patrinos A. The human genome project: Lessons from large-scale biology. Science. 2003;**300**:286-290

[4] Green ED, Watson JD, Collins FS. Human genome project: Twenty-five years of big biology. Nature. 2015;**526**:29-31

[5] Hood L, Rowen L. The human genome project: Big science transforms biology and medicine. Genome Medicine. 2013;**5**:79

[6] Wilson BJ, Nicholls SG. The human genome project, and recent advances in personalized genomics. Risk Manag Healthc Policy. 2015;**8**:9-20

[7] Cho, J., Ahn, S., Son, D. S., Kim, N. K., et al., Bridging genomics and phenomics of gastric carcinoma. International journal of cancer. Journal international du cancer 2019, 145, 2407-2417.

[8] Davatzikos, C., Rathore, S., Bakas, S., Pati, S., et al., Cancer imaging phenomics toolkit: Quantitative imaging analytics for precision diagnostics and predictive modeling of clinical outcome. J Med Imaging (Bellingham) 2018, 5, 011018.

[9] Harder N, Athelogou M, Hessel H, Brieu N, et al. Tissue phenomics for prognostic biomarker discovery in low- and intermediate-risk prostate cancer. Scientific Reports. 2018;**8**:4470

[10] Bai Y, Zhao H. Liquid biopsy in tumors: Opportunities and challenges. Annals of translational medicine. 2018;**6**:S89

[11] Temilola DO, Wium M, Coulidiati TH, Adeola HA, et al. The Prospect and challenges to the flow of liquid biopsy in Africa. Cell. 2019;**8**

[12] Jacobson, R. A., Munding, E., Hayden, D. M., Levy, M., et al., Evolving clinical utility of liquid biopsy in gastrointestinal cancers. Cancers 2019, 11.

[13] Palmirotta, R., Lovero, D., Cafforio, P., Felici, C., et al., Liquid biopsy of cancer: A multimodal diagnostic tool in clinical oncology. Therapeutic advances in medical oncology 2018, 10, 1758835918794630.

[14] Wu J, Hu S, Zhang L, Xin J, et al. Tumor circulome in the liquid biopsies for cancer diagnosis and prognosis. Theranostics. 2020;**10**:4544-4556

[15] Chen L, Chen Y, Feng YL, Zhu Y, et al. Tumor circulome in the liquid biopsies for digestive tract cancer diagnosis and prognosis. World Journal of Clinical Cases. 2020;**8**:2066-2080

[16] Bracht JWP. Mayo-de-Las-casas, C., Berenguer, J., Karachaliou, N., Rosell, R., correction to: The present and future of liquid biopsies in non-small cell lung cancer: Combining four biosources for diagnosis, prognosis, prediction, and disease monitoring. Current Oncology Reports. 2020;**22**:52

[17] De Rubis G, Rajeev Krishnan S, Bebawy M. Liquid biopsies in cancer diagnosis, monitoring, and prognosis. Trends in Pharmacological Sciences. 2019;**40**:172-186

[18] Saarenheimo J, Eigeliene N, Andersen H, Tiirola M, Jekunen A. The value of liquid biopsies for guiding therapy decisions in non-small cell lung cancer. Frontiers in Oncology. 2019;**9**:129

[19] Nair S, Tang KD, Kenny L, Punyadeera C. Salivary exosomes as potential biomarkers in cancer. Oral Oncology. 2018;**84**:31-40

[20] Cheshmi B, Cheshomi H. Salivary exosomes: Properties, medical applications, and isolation methods. Molecular Biology Reports. 2020

[21] Zlotogorski-Hurvitz A, Dayan D, Chaushu G, Korvala J, et al. Human saliva-derived exosomes: Comparing methods of isolation. The Journal of Histochemistry and Cytochemistry. 2015;**63**:181-189

[22] Han Y, Jia L, Zheng Y, Li W. Salivary exosomes: Emerging roles in systemic disease. International Journal of Biological Sciences. 2018;**14**:633-643

[23] Cheng J, Nonaka T, Wong DTW. Salivary exosomes as nanocarriers for cancer biomarker delivery. Materials. 2019;**12**

[24] Nonaka T, Wong DTW. Saliva-exosomics in cancer: Molecular characterization of cancer-derived exosomes in Saliva. Enzyme. 2017;**42**:125-151

[25] Zhan C, Yang X, Yin X, Hou J. Exosomes and other extracellular vesicles in oral and salivary gland cancers. Oral Diseases. 2020;**26**:865-875

[26] Wang ZY, Li F, Rufo J, Chen CY, et al. Acoustofluidic salivary Exosome isolation a liquid biopsy compatible approach for human papillomavirus-associated oropharyngeal cancer detection. Journal of Molecular Diagnostics. 2020;**22**:50-59

[27] A review of saliva: normal composition, flow, and function. *Humphrey SP, Williamson RT J Prosthet Dent. 2001 Feb; 85(2):162-9.*

[28] Structure and biosynthesis of human salivary mucins. Zalewska A, Zwierz K. Zółkowski K, Gindzieński A Acta Biochim Pol. 2000;**47**(4):1067-1079

[29] Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. Edgar WM Br Dent J. 1990 Aug 11-25; 169(3-4):96-8.

[30] Tiwari M. Science behind human saliva. Journal of natural science, biology, and medicine. 2011;**2**(1):53-58

[31] Cove-Smith R, Defective Saliva MA. Proceedings of the Royal Society of Medicine. 1935;**29**:126-127

[32] Farnaud SJ, Kosti O, Getting SJ, Renshaw D. Saliva: Physiology and diagnostic potential in health and disease. The Scientific World Journal. 2010;**10**:434-456

[33] Humphrey SP, Williamson RT. A review of saliva: Normal composition, flow, and function. The Journal of prosthetic dentistry. 2001 Feb 1;**85**(2):162-169

[34] Holsinger F, Bui D. Salivary Gland Disorders. Berlin, Germany: Springer; 2007

[35] Pharmacokinetic principles of drug distribution in saliva. Jusko WJ, Milsap RL. Annals of the New York Academy of Sciences. 1993 Sep 20;**694**:36-47

[36] The application of saliva, sweat and tear fluid for diagnostic purposes. Haeckel R, Hänecke P Ann Biol Clin (Paris). 1993;**51**(10-11):903-910

[37] Nagarathinam AE, Kumar TD, Kumar AR, Vasanthira K, Lakshmi RS,

Jayant VS. Salivary Urea and Creatinine as a Diagnostic Marker of Chronic Kidney Disease – Review. (IOSR-JDMS). 2017;**16**:95-100

[38] Schneyer LH, Schneyer CA. Secretion of Saliva. Advances in Oral Biology. 1964;**1**:1-31

[39] Leung SW. A demonstration of the importance of bicarbonate as a salivary buffer. Journal of Dental Research. 1951;**30**:403-414

[40] Lee JM, Garon E, Wong DT. Salivary diagnostics. Orthodontics & Craniofacial Research. 2009;**12**:206-211

[41] Hundt S, Haug U, Brenner H. Blood markers for early detection of colorectal cancer: A systematic review. Cancer Epidemiology and Prevention Biomarkers. 2007 Oct 1;**16**(10):1935-1953

[42] De Seny D, Fillet M, Meuwis MA, Geurts P, Lutteri L, Ribbens C, et al. Discovery of new rheumatoid arthritis biomarkers using the surface-enhanced laser desorption/ionization time-of-flight mass spectrometry ProteinChip approach. Arthritis and Rheumatism. 2005 Dec;**52**(12):3801-3812

[43] Hodgetts A, Levin M, Kroll JS, Langford PR. Biomarker discovery in infectious diseases using SELDI. Future Microbiology. 2007;**2**(1):35-49

[44] Suzuki M, Ross GF, Wiers K, et al. Identification of a urinary proteomic signature for lupus nephritis in children. Pediatric Nephrology. 2007;**22**(12):2047-2057

[45] C. S. Buhimschi, V. Bhandari, B. D. Hamar et al., "Proteomic profiling of the amniotic fluid to detect inflammation, infection, and neonatal sepsis," PLoS Medicine, vol. 4, no. 1, article e18, 2007.

[46] De Bock M, De Seny D, Meuwis MA, Chapelle JP, Louis E. Malaise M. Fillet M. Challenges for biomarker discovery in body fluids using SELDI-TOF-MS. Journal of Biomedicine and Biotechnology: Merville MP; 2009 Dec 6. p. 2010

[47] Malamud D. PhDa,b and Isaac R. Rodriguez-Chavez, PhDc, Saliva as a diagnostic fluid. Published in final edited form as: Dent Clin North Am. 2011 January;**55**(1):159-178

[48] Campo J, Perea MA, Del Romero J, Cano J, Hernando V, Bascones A. Oral transmission of HIV, reality or fiction? An update. Oral Diseases. 2006;**12**:219-228

[49] Wei F, Yang J, Wong DT. Detection of exosomal biomarker by electric field-induced release and measurement (EFIRM). Biosensors & Bioelectronics. 2013;**44**:115-121

[50] Wei F, Lin CC, Joon A, Feng Z, Troche G, Lira ME, et al. Noninvasive saliva-based EGFR gene mutation detection in patients with lung cancer. American Journal of Respiratory and Critical Care Medicine. 2014;**190**:1117-1126

[51] Han Y, Jia L, Zheng Y, Li W. Salivary exosomes: Emerging roles in systemic disease. International Journal of Biological Sciences. 2018;**14**:633-643

[52] Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cellular and molecular life sciences : CMLS. 2018;**75**:193-208

[53] Baixauli F, Lopez-Otin C, Mittelbrunn M. Exosomes and autophagy: Coordinated mechanisms for the maintenance of cellular fitness. Frontiers in Immunology. 2014;**5**:403

[54] Lai R, Yeo R, Tan K, Lim S. Exosomes for drug delivery – A novel application for the mesenchymal stem cell. Biotech Adv. 2013;**31**:543-551

[55] Zheng X, CHEN F, ZHANG J, ZHANG Q, Exosome Analysis LINJ. A promising biomarker system with special attention to saliva. The Journal of Membrane Biology. 2014;**247**:1129-1136

[56] Johnstone RM. Revisiting the road to the discovery of exosomes. Blood Cells, Molecules & Diseases. 2005;**34**:214-219

[57] Simons M, Raposo G. Exosomes - vesicular carriers for intercellular communication. Current Opinion in Cell Biology. 2009;**21**:575-581

[58] Valadi H, Ekstrom K, Bossios A, Sjostrand M, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology. 2007;**9**:654-U672

[59] Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: Database of exosomal proteins, RNA and lipids. Nucleic Acids Research. 2012;**40**:D1241-D1244

[60] Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles. and friends. The Journal of cell biology. 2013;**200**:373-383

[61] Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids. 2014;**1841**:108-120

[62] Becker A, Thakur BK, Weiss JM, Kim HS, et al. Extracellular vesicles in cancer: Cell-to-cell mediators of metastasis. Cancer Cell. 2016;**30**:836-848

[63] Costa-Silva, B., Aiello, N. M., Ocean, A. J., Singh, S., et al., Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol 2015, 17, 816−+.

[64] Hoshino, A., Costa-Silva, B., Shen, T. L., Rodrigues, G., et al., Tumour exosome integrins determine organotropic metastasis. Nature 2015, 527, 329−+.

[65] Kharaziha P, Ceder S, Li Q, Panaretakis T. Tumor cell-derived exosomes: A message in a bottle. Bba-Rev Cancer. 2012;**1826**:103-111

[66] Guo Q, Jiang C. Delivery strategies for macromolecular drugs in cancer therapy. Acta Pharmaceutica Sinica B. 2020;**10**:979-986

[67] Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. Clinica Chimica Acta. 2007;**383**:30-40

[68] Yang, J., Wei, F., Schafer, C., Wong, D. T., Detection of tumor cell-specific mRNA and protein in exosome-like microvesicles from blood and saliva. PLoS One 2014, 9, e110641.

[69] Zhou HX, Xu WR, Qian H, Yin Q, et al. Circulating RNA as a novel tumor marker: An in vitro study of the origins and characteristics of extracellular RNA. Cancer Letters. 2008;**259**:50-60

[70] Aro K, Kaczor-Urbanowicz K, Carreras-Presas CM. Salivaomics in oral cancer. Current Opinion in Otolaryngology & Head and Neck Surgery. 2019;**27**:91-97

[71] Winck FV, Prado Ribeiro AC, Ramos Domingues R, Ling LY, Riaño-Pachón DM, Rivera C, et al. Insights into immune responses in oral cancer through proteomic analysis of saliva and salivary extracellular vesicles. Scientific Reports. 2015;**5**:16305

[72] Li L, Li C, Wang S, Wang Z, Jiang J, Wang W, et al. Exosomes derived from hypoxic oral squamous cell carcinoma cells deliver miR-21 to normoxic cells to elicit a prometastatic phenotype. Cancer research. 2016 Apr 1;**76**(7):1770-1780

[73] Sakha S, Muramatsu T, Ueda K, Inazawa J. Exosomal microRNA miR-1246 induces cell motility and invasion through the regulation of DENND2D in oral squamous cell carcinoma. Scientific Reports. 2016 Dec 8;**6**:38750

[74] Kawakubo-Yasukochi T, Morioka M, Hazekawa M, Yasukochi A, Nishinakagawa T, Ono K. miR-200c-3p spreads invasive capacity in human oral squamous cell carcinoma microenvironment. Molecular Carcinogenesis. 2017

[75] Li YY, Tao YW, Gao S, Li P, Zheng JM, Zhang SE, et al. Cancer-associated fibroblasts contribute to oral cancer cells proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. eBioMedicine. 2018 Oct 1;**36**:209-220

[76] Li C, Zhou Y, Liu J, Su X, Qin H, Huang S, et al. Potential markers from serum-purified exosomes for detecting oral squamous cell carcinoma metastasis. Cancer Epidemiology and Prevention Biomarkers. 2019 Oct 1;**28**(10):1668-1681

[77] Xie C, Du LY, Guo F, Li X, Cheng B. Exosomes derived from microRNA-101-3p-overexpressing human bone marrow mesenchymal stem cells suppress oral cancer cell proliferation, invasion, and migration. Molecular and Cellular Biochemistry. 2019 Aug 15;**458**(1-2):11-26

[78] Sun LP, Xu K, Cui J, Yuan DY, Zou B, Li J, et al. Cancer-associated fibroblast-derived exosomal miR-382-5p promotes the migration and invasion of oral squamous cell carcinoma. Oncology reports. 2019 Oct 1;**42**(4):1319-1328

[79] He L, Ping F, Fan Z, Zhang C, Deng M, Cheng B, Xia J. Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. Biomedicine & Pharmacotherapy. 2020 Jan 1;121:109553.

[80] Chen JH, Wu AT, Bamodu OA, Yadav VK, Chao TY, Tzeng YM, et al. Ovatodiolide suppresses oral cancer malignancy by down-regulating exosomal mir-21/STAT3/β-catenin cargo and preventing oncogenic transformation of normal gingival fibroblasts. Cancers. 2020 Jan;**12**(1):56

[81] Kirave P, Gondaliya P, Kulkarni B, Rawal R, Garg R, Jain A, et al. Exosome mediated miR-155 delivery confers cisplatin chemoresistance in oral cancer cells via epithelial-mesenchymal transition. Oncotarget. 2020;**11**(13):1157-1171 https://doi.org/10.18632/oncotarget.27531

[82] Yeh CK, Christodoulides NJ, Floriano PN, Miller CS, et al. Current development of saliva/oral fluid-based diagnostics. Texas Dental Journal. 2010;**127**:651-661

[83] Yan W, Apweiler R, Balgley BM, Boontheung P, et al. Systematic comparison of the human saliva and plasma proteomes. Proteomics. Clinical Applications. 2009;**3**:116-134

[84] Ohshiro K, Rosenthal DI, Koomen JM, Streckfus CF, et al. Pre-analytic saliva processing affect proteomic results and biomarker screening of head and neck squamous carcinoma. International Journal of Oncology. 2007;**30**:743-749

[85] Hu S, Arellano M, Boontheung P, Wang JH, et al. Salivary proteomics for Oral cancer biomarker discovery. Clinical Cancer Research. 2008;**14**:6246-6252

[86] Dowling P, Wormald R, Meleady P, Henry M, et al. Analysis of the saliva proteome from patients with head and neck squamous cell carcinoma reveals differences in abundance levels of proteins associated with tumour progression and metastasis. Journal of Proteomics. 2008;**71**:168-175

[87] Rai B, Kaur J, Jacobs R, Anand SC. Adenosine deaminase in saliva as a diagnostic marker of squamous cell carcinoma of tongue. Clin Oral Invest. 2011;**15**:347-349

[88] Elashoff D, Zhou H, Reiss J, Wang JH, et al. Prevalidation of salivary biomarkers for Oral cancer detection. Cancer Epidem Biomar. 2012;**21**:664-672

[89] Winck FV, Prado Ribeiro AC, Ramos Domingues R, Ling LY, Riaño-Pachón DM, Rivera C, et al. Insights into immune responses in oral cancer through proteomic analysis of saliva and salivary extracellular vesicles. Scientific Reports. 2015;**5**:16305

[90] Zlotogorski-Hurvitz A, Dayan D, Chaushu G, Salo T, Vered M. Morphological and molecular features of oral fluid-derived exosomes: Oral cancer patients versus healthy individuals. Journal of Cancer Research and Clinical Oncology. 2016;**142**:101-110

[91] Ludwig S, Sharma P, Theodoraki MN, Pietrowska M, et al. Molecular and functional profiles of exosomes from HPV(+) and HPV(−) head and neck cancer cell lines. Frontiers in Oncology. 2018;**8**

[92] He L, Ping F, Fan ZN, Zhang C, et al. Salivary exosomal miR-24-3p serves as a potential detective biomarker for oral squamous cell carcinoma screening. Biomedicine & Pharmacotherapy. 2020;**121**

[93] Sakha S, Muramatsu T, Ueda K, Inazawa J. Exosomal microRNA miR-1246 induces cell motility and invasion through the regulation of DENND2D in oral squamous cell carcinoma. Scientific Reports. 2016;**6**

[94] Li L, Li C, Wang S, Wang Z, et al. Exosomes derived from hypoxic Oral squamous cell carcinoma cells deliver miR-21 to normoxic cells to elicit a Prometastatic phenotype. Cancer Research. 2016;**76**:1770-1780

[95] Valadi H, Ekstrom K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology. 2007;**9**:654-659

[96] Skog J, Wurdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nature Cell Biology. 2008;**10**:1470-1476

[97] Hong BS, Cho JH, Kim H, et al. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. BMC Genomics. 2009;**10**:556

[98] Li Y, John MA, Zhou X, Kim Y, Sinha U, Jordan RC, et al. Salivary transcriptome diagnostics for oral cancer detection. Clinical Cancer Research. 2004 Dec 15;**10**(24):8442-8450

[99] Ludwig N, Yerneni SS, Razzo BM, Whiteside TL. Exosomes from hnscc promote angiogenesis through reprogramming of endothelial cells. Molecular Cancer Research. 2018;**24**:24. DOI: 10.1158/1541-7786.MCR-18-0358

[100] Xiao C, Song F, Zheng YL, Lv J, et al. Exosomes in head and neck squamous cell carcinoma. Frontiers in Oncology. 2019;**9**:894

[101] Ludwig N, Gillespie DG, Reichert TE, Jackson EK, Whiteside TL. Purine metabolites in tumor-derived exosomes may facilitate immune escape of head and neck squamous cell carcinoma. Cancers. 2020;**12**

[102] Jelonek K, Wojakowska A, Marczak L, Muer A, et al. Ionizing radiation affects protein composition of

exosomes secreted in vitro from head and neck squamous cell carcinoma. Acta Biochimica Polonica. 2015;**62**:265-272

[103] Ebnoether E, Muller L. Diagnostic and therapeutic applications of exosomes in cancer with a special focus on head and neck squamous cell carcinoma (HNSCC). International Journal of Molecular Sciences. 2020;**21**

Chapter 3

Cytopathology of Intraoral Salivary Gland Tumours and Tumour-Like Lesions: Diagnostic Challenges and Pitfalls

Jai Kumar Chaurasia and Neelkamal Kapoor

Abstract

Fine needle aspiration cytology is an important diagnostic tool in cytopathology. There are many challenges and pitfalls encountered in intraoral salivary gland cytopathology as tumours of these glands show morphological diversity and overlapping features. There are often variable solid-cystic components, metaplastic or necrotic changes, fibrosis, hyalinisation and haemorrhage accounting for heterogeneity of these tumours. The tumour profile of intraoral salivary gland is quite different from the major salivary glands and needs special attention. A low-grade malignant tumour may sometimes mimic a benign neoplasm or a non-neoplastic lesion resulting in a false negative diagnosis. Moreover, misinterpretation and failure to recognize subtle morphological and architectural patterns of cells also pose diagnostic challenges. In this chapter, we intend to highlight the key cytopathological features of intraoral salivary gland tumours and tumour-like lesions with emphasis to overcome diagnostic challenges and pitfalls to avoid misdiagnosis which will aid in planning further management and treatment.

Keywords: Challenges, Cytopathology, Intraoral, Pitfalls, Salivary

1. Introduction

Fine needle aspiration cytology (FNAC) is an important diagnostic tool for initial evaluation of salivary gland (SG) tumours. The global annual incidence for all SG tumours varies from 0.4 to 13.5 cases per 100,000 population [1]. Tumours of intraoral salivary glands (SGs) are relatively uncommon as compared to tumours of major SGs and constitutes 10–15% of all SG tumours with relative frequency varying from 0.4% to 1.52% in different part of the world [2, 3]. These tumours have a very distinct profile from tumours of major SGs with reference to tumour histological type, clinical presentation and distribution. Majority of intraoral minor SG tumours are malignant in contrast to major SG tumours where benign tumours outnumber the malignant ones [4]. While some studies documents mucoepidermoid carcinoma (MEC) as the most common malignant tumour of minor SGs, other studies documents adenoid cystic carcinoma (ADCC) or pleomorphic adenoma (PA) as the most common tumour [5–10]. This difference in frequency of these histological types may be attributed to the differences in geographic location, race and varied

clinical presentation. The clinical presentation of intraoral SG tumours also range from mild pain to visible palpable mass in the oral cavity with or without ulceration leading to obstructive features such as difficulty in swallowing and deglutition. These tumours can occur at various intraoral locations such as mucosa of lips and cheeks, hard and soft palate, uvula, floor of the mouth, tongue, retromolar area and peritonsillar region. The hard palate is the most common site of occurrence [3–5]. Cytopathological evaluation of intraoral SG tumours is challenging as these tumours show heterogeneity and considerable morphological diversity and overlap [6]. The correct preoperative cytopathological diagnosis of intraoral SG tumours is essential for deciding further course of management and treatment. We here present key cytomorphological features of intraoral salivary gland tumours and tumour-like lesions with emphasis to overcome diagnostic challenges and pitfalls.

2. FNAC: a vital diagnostic tool in salivary gland pathology

FNAC is easy, minimally invasive, cost effective technique which provides a rapid initial preoperative diagnosis of SG tumours and has an impact on subsequent management and treatment [11]. The sensitivity and specificity of FNAC in diagnosing SG tumours is 85–100% and 90–100% respectively. The aspirated material obtained through FNAC can also be utilized for special staining such as Periodic acid-Schiff (PAS), Periodic acid-Schiff with diastase (PAS-D), Mucicarmine, Phosphotungstic acid-haematoxylin (PTAH), Acid fast bacilli (AFB) and Gram staining for further evaluation and diagnosis. PAS-D and mucicarmine are particularly useful for highlighting mucin containing cells in challenging cases of low-grade mucoepidermoid carcinoma (MEC). Similarly, PTAH stain can be applied on paraffin-embedded cell block preparation for identification oncocytic cells in challenging diagnosis of tumours with oncocytic differentiation. The cell blocks can also be utilized for Immunohistochemistry (IHC) for demonstrating epithelial and myoepithelial components in diagnosis of challenging tumours. The epithelial cells are positive for immunohistochemical markers such as cytokeratin and epithelial membrane antigen (EMA) and the myoepithelial cells show positivity for smooth muscle actin (SMA), calponin, p63 and S-100. Further, the aspirated material can also be used for microbiological culture, immunophenotyping and molecular analysis for confirming the cytological diagnosis. While the FNAC of palpable lesions in major SGs is relatively easy, the FNAC of intraoral SGs is challenging as many times aspirates are not cellular as these intraoral sites are often difficult to approach and sometimes inaccessible [10–12]. In such cases, radiological-guided FNAC may be advised for better yield of aspirates for subsequent cytological diagnosis. Also, while performing FNAC, there are chances of complications such as hemorrhage, nerve pain and damage and infection. There can be post FNAC induced changes in tissue such as squamous metaplastic changes, inflammation, granulation tissue formation and sometimes infarction, which may interfere with subsequent histological diagnosis. Therefore, familiarity with key cytological features with recognition of the subtle cytomorphological changes in cells is crucial for overcoming barriers and making a correct diagnosis.

3. The Milan system for reporting salivary gland cytopathology

The Milan system for reporting salivary gland cytopathology (MSRSGC) is an evidence based international classification and was developed by international consortium with the aim to standardize reporting terminology for categorizing SG lesions [13]. The intraoral SG tumour and tumour-like lesions can also be

categorized according to MSRSGC. The system has advantage and impact on clinical management of the patients [13, 14]. The system consists of following six broad diagnostic categories.

3.1 Category I: non-diagnostic

It includes FNAC smears with insufficient cellular material for making a definite diagnosis.

3.2 Category II: non-neoplastic

Includes FNAC smears with benign non-neoplastic lesions such as sialadenitis, sialolithiasis, granulomatous inflammation etc.

3.3 Category III: atypia of undetermined significance (AUS)

FNAC smears with limited cellular atypia that lacks qualitative or quantitative features of a neoplasm. Smears showing reactive or reparative atypia and metaplastic changes are included in this category.

3.4 Category IV

This category is classified into**:**

Category IVA: Benign Neoplasm – FNAC specimens showing characteristic cytomorphological features of a benign epithelial neoplasm.

Category IVB: Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP) – This category is most challenging as it includes FNAC specimens that have diagnostic feature of a neoplasm however, the possibility of malignant neoplasm cannot be excluded.

3.5 Category V: suspicious of malignancy

FNAC specimens that have some but not all the criteria for a specific diagnosis of malignancy and yet the overall cytologic features are sugsgestive of malignancy.

3.6 Category: VI malignant

FNAC specimens that are diagnostic of malignancy. The tumours of intraoral SGs with frank features of malignancy are included in this category.

4. Overview

In this chapter, the intraoral SG tumours and tumour-like lesions are being discussed under following six headings for better understanding of these tumours and related diagnostic challenges and pitfalls.

4.1 Matrix-containing tumours

The matrix producing tumours of intraoral SGs are pleomorphic adenoma (PA), adenoid cystic carcinoma (ADCC), polymorphous adenocarcinoma (POA). Carcinoma ex pleomorphic adenoma (CEPA), epithelial-myoepithelial carcinoma (EMC) are other matrix producing tumours that can also occur in intraoral SGs.

4.1.1 Pleomorphic adenoma (PA)

Pleomorphic adenoma is the most common benign tumour of SGs. Although, majority of PA occur in parotid gland, some studies documents PA as most common neoplasm in intraoral minor SGs [2, 4]. In the oral cavity, it usually presents as a solitary nodule or mass in the palate, sometimes with obstructive clinical symptoms.

Key cytological features – Smears show variable cellularity with cells arranged in clusters and sheets, embedded in a fibrillary chondromyxoid ground substance or matrix (**Figure 1**). The majority of cells are myoepithelial which may be ovoid, spindle, plasmacytoid, epithelioid, clear or stellate shaped. The nuclei of cells are round to oval, often with eccentric nucleus with bland finely granular chromatin and inconspicuous nucleoli. The cytoplasm is pale with well-defined cell borders. Stripped naked nuclei are not seen. PA may show diverse metaplastic changes including squamous metaplasia with or without keratinisation, oncocytic, clear cell, sebaceous, lipomatous and cartilaginous metaplasia. Hyaline globules can be seen. The prototypical PA is placed in benign category (IVA) of the Milan system.

Diagnostic challenges and pitfalls – Pleomorphic Adenoma (PA)

a. Sometimes, aspirate from PA may consist of only chondromyxoid substance without presence of epithelial and myoepithelial cells. The diagnosis of a neoplasm can be suspected but reaspiration is needed to rule out the possibility of other matrix producing tumours such as adenoid cystic carcinoma (ADCC) which can also occur at various intraoral locations. Both of these tumours have fibrillar metachromatic matrix and may show formation of hyaline globules. However, the matrix in PA is fibrillar with frayed edges (**Figure 2a**) while in ADCC the matrix is in form of beaded finger-like fragments, acellular spheres and tubules with sharply defined edges (**Figure 2b**) [15, 16]. Cellular PA with scant matrix particularly may resemble solid variant of ADCC. The cells of ADCC are basaloid and careful examination of nuclear chromatin of cells reveals distinguishing features. The cells in PA have bland finely granular chromatin while the cells of ADCC have coarse nuclear chromatin, high nuclear-cytoplasmic (N:C) ratio, scant cytoplasm and nucleoli [15]. The cells of ADCC may show focal nuclear moulding. Stripped naked nuclei can be seen in ADCC but not in PA. However, in challenging cases, it is not always possible to distinguish between the two entities. Such cases may be placed in SUMP category (IVB) of the Milan system. Follow-up and excision may be advised keeping clinical context in mind.

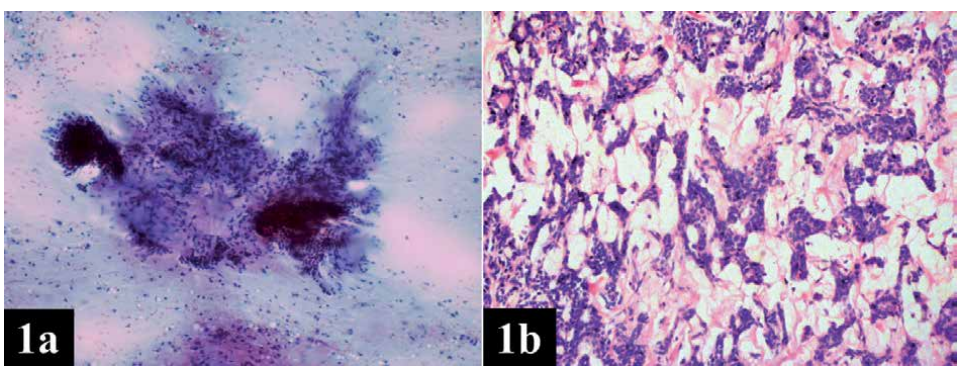

Figure 1.
1a: FNAC smear showing abundant chondromyxoid matrix with embedded cells in a case of pleomorphic adenoma (May-Grunwald Giemsa stain x 40), 1b: Corresponding histopathology showing nests, interlacing cords and strands of epithelial and myoepithelial cells in a myxoid stroma (Haematoxylin & Eosin x 40).

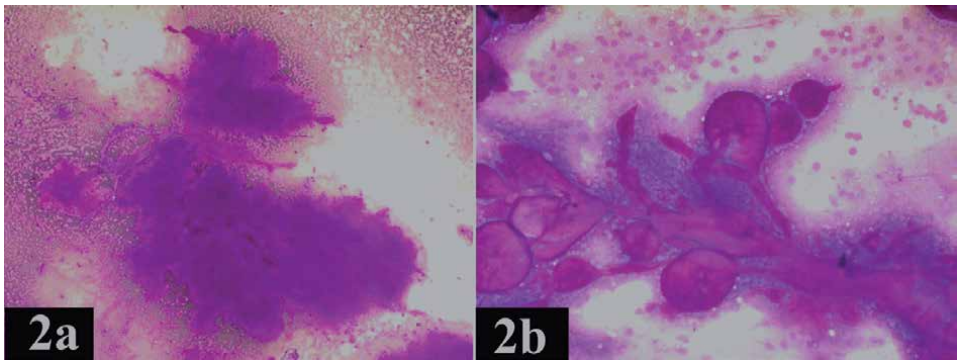

Figure 2.
2a: Smear showing abundant fibrillar metachromatic matrix with frayed edges in pleomorphic adenoma (May-Grunwald Giemsa stain x 40), 2b: Showing metachromatic matrix in form of acellular beaded, finger-like fragments and hyaline spherical globules with well-defined borders in a case of adenoid cystic carcinoma (May-Grunwald Giemsa stain x 40).

b. Cells in PA may undergo cystic and metaplastic changes. The aspirate may consist of metaplastic squamous cells with or without keratinisation (**Figure 3**) and scant or absent fibrillar metrachromatic matrix which can raise suspicion of a low-grade mucoepidermoid carcinoma (MEC) [16]. The intermediate cells of MEC in particular resemble squamous metaplastic cells of PA. However, the MEC usually have a dirty background and lacks myoepithelial cells, chondromyxoid material and keratinisation. One should also search for mucus cells, if MEC is suspected. In challenging cases, mucin containing cells of MEC can be demonstrated by using PAS-D or mucicarmine staining on cell blocks.

c. The myoepithelial cells in PA may sometimes show reactive atypia and prominent anisokaryosis which may sometimes be difficult to distinguish from malignant tumours [17]. Cells with reactive atypia can be recognized as they have bland chromatin and inconspicuous mitotic activity. The diagnostic pitfall is the occurrence of carcinoma ex-pleomorphic adenoma (CEPA). The development of CEPA in pre-existing intraoral PA is rare but few cases have been reported in literature [18]. History of long standing or recurrent PA in oral cavity with recent sudden increase in size along with cytological evidence

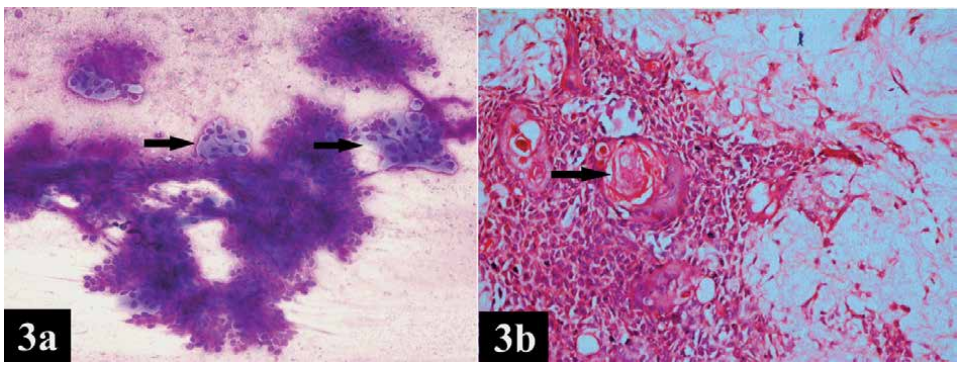

Figure 3.
3a: FNAC smear showing pleomorphic adenoma with squamous metaplasia (arrows) (May-Grunwald Giemsa stain x 4), 3b: Corresponding histology showing squamous metaplasia with evidence of keratinisation (arrow) surrounded by myxoid stroma (Haematoxylin & Eosin x 40).

of high grade atypical features in cells (**Figure 4**) and even a focal presence of areas depicting conventional PA consisting of benign cell clusters and chondromyxoid matrix (**Figure 4**), should lead to the diagnosis of CEPA [18, 19].

d. Epithelial-myoepithelial carcinoma (EMC) may sometimes also simulate a PA. It is a an unusual tumour and can also occur in minor SGs and palate [20]. This tumour can also have hyaline globules and show thin basement membrane-like metachromatic material around the clusters of cells. The predominant cell of EMC is myoepithelial. These cells are larger with abundant fragile clear delicate cytoplasm. Unlike PA, naked or stripped nuclei can be seen. Moreover, the biphasic population of epithelial and myoepithelial cells can be identified in EMC. The key cytological features and pitfalls of EMC are discussed at 4.3.1.

e. PA with predominant myoepithelial component (**Figure 5a**) may resemble other myoepithelial cell containing tumours such as myoepithelioma/myoepithelial adenoma (**Figure 5b**) in oral cavity. Myoepithelioma lacks chondroid stroma and duct cells seen of PA [21]. Distinction may not always be possible

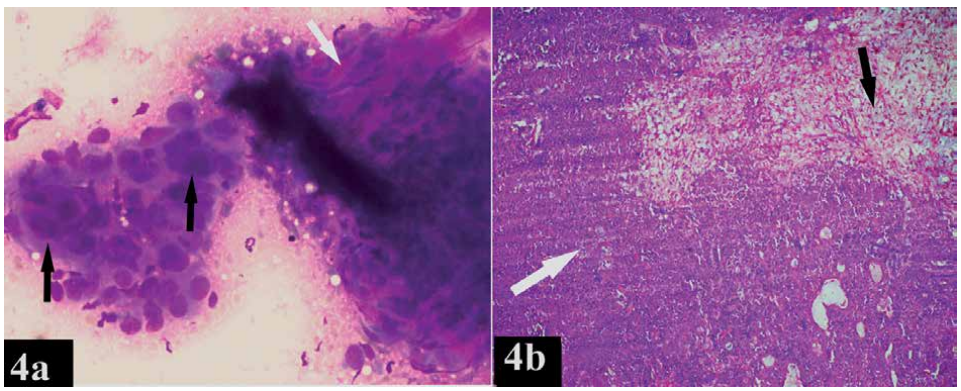

Figure 4.
4a: Showing malignant cells with high N:C ratio and hyperchromatic nuclei (black arrows) in a case of carcinoma ex-pleomorphic adenoma with evidence of metachromatic matrix of pre-existing pleomorphic adenoma (white arrow) (May-Grunwald Giemsa stain x 40), 4b: Corresponding histology showing sheet of malignant cells (white arrow) arising in background of myxoid stroma (black arrow) of pleomorphic adenoma (Haematoxylin & Eosin x 10).

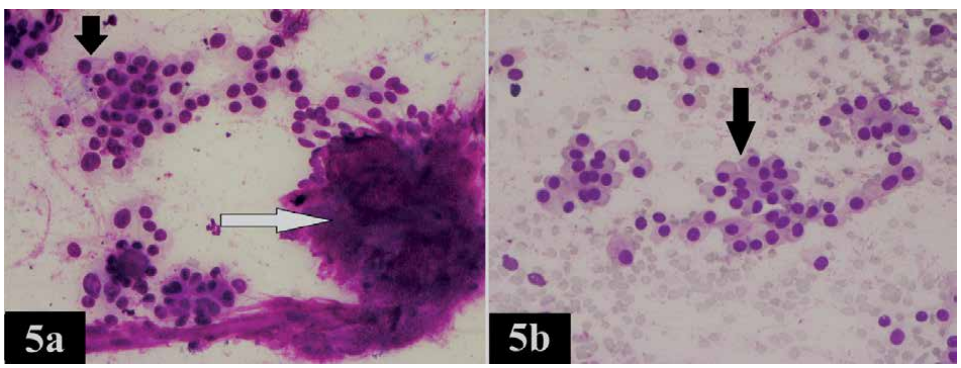

Figure 5.
5a: FNAC smear showing population of myoepithelial cells (black arrow) with metachromatic matrix (white arrow) in a case of myoepithelial predominant pleomorphic adenoma (May-Grunwald Giemsa stain x 40). 5b: Smear showing myoepithelial cells with pale to clear cytoplasm (arrow) in a case of a myoepithelioma (May-Grunwald Giemsa stain x 40).

and is not of much significance as both entities are benign (Milan system category IVA) and management is almost similar.

4.1.2 Adenoid cystic carcinoma (ADCC)

It constitutes nearly 10% of all SG tumours. Some studies suggest it to be the most common malignancy in minor salivary gland with hard palate being the most common site [22–24].

Key cytological features – Smears are cellular and cells are seen in clusters showing overlapping and multilayering. Tumour cells are also seen arranged in cup-shaped fragments and also scattered singly (**Figure 6**). The cells are basaloid with scant cytoplasm, round to ovoid hyperchromatic nuclei with coarse nuclear chromatin and nucleoli. The metachromatic matrix is in form of acellular beaded, finger like fragments, branching tubules and also forms hyaline spherical globules with well-defined borders (**Figure 2b**).

Diagnostic challenges and pitfalls – ***Adenoid Cystic Carcinoma (ADCC)***

a. PA and its distinguishing features from ADCC are discussed at 4.1.1a. However, the diagnosis of solid variant of ADCC is challenging as it lacks metachromatic matrix [25]. It can be distinguished from PA as it shows three dimensional clusters of basaloid cells with variable degree of pleomorphism. The cells are more hyperchromatic and angulated than in PA. In contrast to PA, mitosis, apoptosis and necrosis can also be seen [25]. IHC on cell blocks with CD -117 can be done in challenging cases which shows strong cytoplasmic positivity in ADCC [25, 26].

b. Sometimes, the basement membrane-like metachromatic material in EMC forms large globules resembling ADCC but the cellular compartment is entirely different in ADCC [26]. The cells of ADCC are basaloid with scant cytoplasm with round to ovoid hyperchromatic nuclei with coarse nuclear chromatin and nucleoli in contrast to myoepithelial cells of EMC with clear cytoplasm and round nuclei and vesicular chromatin [26].

c. Basal Cell Adenoma (BCA) can resemble ADCC. However, BCA arise predominantly in parotid and is extremely rare in the intraoral minor SGs [27]. It also shows metachromatic matrix and basement membrane like material and

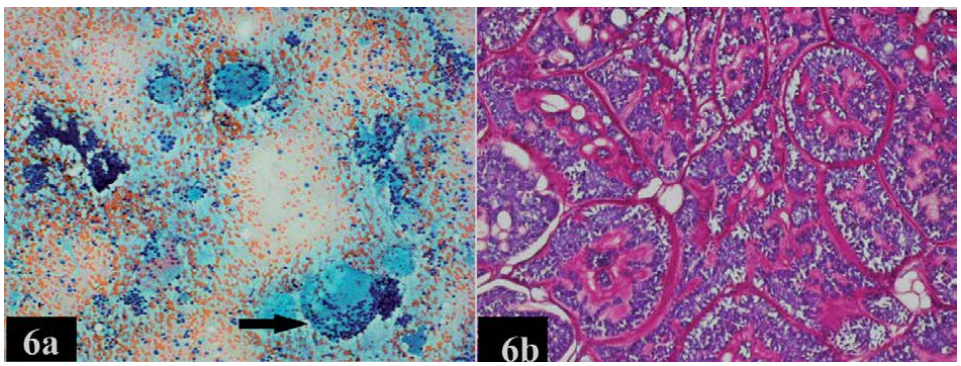

Figure 6.
6a: Smear showing large hyaline spherical globules with well-defined borders with attached tumour cells in cup-shaped fashion (arrow) and also scattered singly in a case of adenoid cystic carcinoma (Papanicolaou stain x 4). 6b: Corresponding histological section showing tumour cells surrounded by basement membrane-like metachromatic material (PAS x 40).

basaloid cells similar to ADCC but cells have granular chromatin rather than coarse nuclear chromatin seen in ADCC. The cells occasionally show peripheral palisading. Squamous morules or metaplasia can be frequently seen in BCA but not in ADCC.

d. Canalicular (CA) /Ductal Adenoma occurs in oral cavity predominantly in upper lip and buccal region [28]. It has overlapping cytological features with BCA. Cells of CA are cuboidal to columnar and are seen in clusters and cords. Unlike ADCC, the cells are monomorphic with finely dispersed chromatin and inconspicuous nucleoli.

4.1.3 Polymorphous adenocarcinoma (POA)

Previously called as polymorphous low grade adenocarcinoma, it is a low-grade matrix containing tumour occurring predominantly in intraoral minor salivary gland with palate being the most common site [29].

Key cytological features – Cells are seen in clusters, papillae, trabeculae, singly scattered and also in form of epithelial fragments attached to fibrovascular stromal cores (**Figure 7**). The cells are monomorphic with oval nuclei, open chromatin and indistinct nucleoli with moderate amount of cytoplasm. Small hyaline globules can be seen admixed with these cells.

Diagnostic challenges and pitfalls – Polymorphous Adenocarcinoma (POA).

The cytological features of POA may resemble PA or ADCC [30]. The matrix of POA may be fibrillar and myxoid resembling a PA or can form hyaline globules similar to ADCC. However, the papillary architecture of cells is found in POA and its presence can distinguish it from PA and ADCC. The cells of ADCC are basaloid with coarse nuclear features rather than fine open nuclear features seen in POA [30].

4.2 Cystic tumours

Cystic tumours of intraoral SGs range from benign tumours such as sclerosing polycystic adenosis, cystadenoma, cystic PA, duct papilloma to malignant tumours such as low-grade MEC and papillary cystic variant of acinic cell carcinoma (AciCC). Evaluation and interpretation of these cystic tumours is particularly challenging as usually the aspirate of these tumours is hypocellular. This may result in false negative diagnosis particularly in a low-grade malignant cystic tumours.

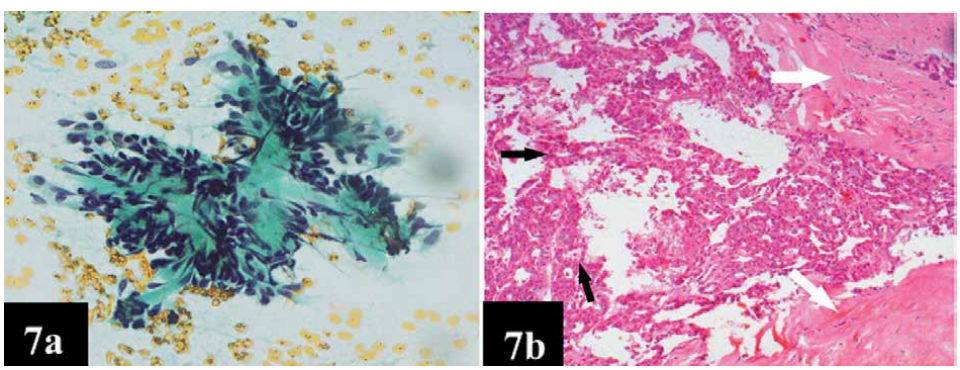

Figure 7.
7a: Smear showing monomorphic small round to oval epithelial cells attached to fibrous stromal core in a case of polymorphous adenocarcinoma (Papanicolaou stain x 40), 7b: Corresponding histology showing growth of tumour cells in glandular and acinar pattern (black arrows) surrounded by adjacent fibrous stroma (white arrows) (Haematoxylin & Eosin x 10).

Diagnostic challenges and Pitfalls – Cystic Tumours

a. **Sclerosing polycystic adenosis (SPA)** is a rare benign tumour reported in intraoral minor SGs [31]. It is predominantly cystic and shares common features with fibrocystic disease and sclerosing adenosis of the breast. The key cytological features include epithelial cells in syncytial sheets with apocrine changes with variable granular to oncocytic and vacuolated cytoplasm in a cystic background consisting of foamy histiocytes and proteinaceous material. SPA is frequently associated with intraductal proliferations. Ductal cells of SPA with vacuolated cytoplasm can resemble cells of AciCC or a low-grade cystic MEC. However, the apocrine metaplastic changes in cells is the key feature which in proper clinical context of long history of benign tumour favours a diagnosis of SPA over other carcinomas [31].

b. **Cystadenoma** is also a rare benign tumour occurring in minor SGs [32]. The tumour is multicystic and cytology reveals cuboidal to columnar cells in clusters and papillae with bland nuclear features and oncocytic cytoplasm. The cytoplasm of cells may sometimes become epidermoid and may resemble cells of low-grade MEC. Cystadenoma is indistinguishable from cystadenomacarcinoma on cytology. Histological evidence of invasion is required for diagnosis of cystadenocarcinoma [32].

c. **Duct papilloma (DP)** is a benign tumour arising predominantly from the ducts of minor SGs and characterized by intraductal proliferation of cells in form of broad papillary projections [33]. It is composed of epidermoid cells and mucous cells and can resemble a MEC. However, the papillary architecture of DP can help to distinguish it from MEC.

d. **Mucoepidermoid carcinoma (MEC)** – It is the most common malignancy of intraoral SGs in young adults and children [7–9]. Palate is the most common site of occurrence. It is composed of mucin containing cells, intermediate cells and epidermoid/squamous cells. The low-grade MEC is cystic and particularly challenging as it mimics other cystic neoplasms and non-neoplastic cystic tumour-like lesions [34]. Aspirates from low-grade MEC show mucin containing vacuolated cells in a predominantly cystic background with debris. However, it can be recognized by presence of at least few or occasional cluster or single intermediate or epidermoid cells (**Figure 8**). Intermediate

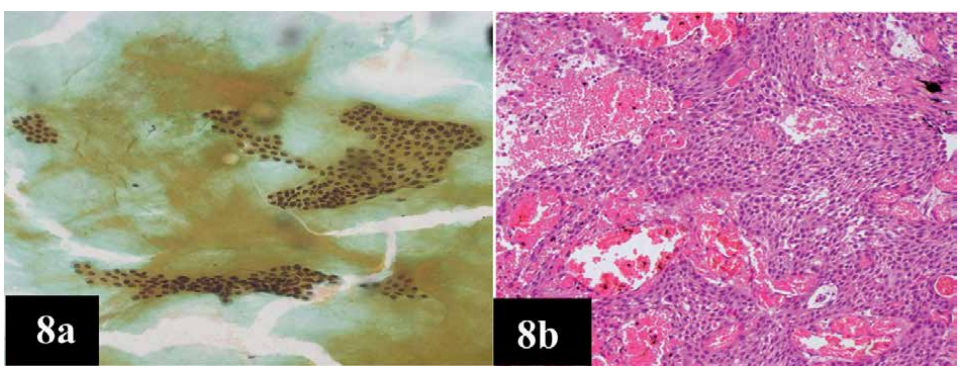

Figure 8.
8a: Showing small sheets and clusters of epidermoid cells in a cystic mucoid background in a case of low-grade mucoepidermoid carcinoma (Papanicolaou stain x 10), 8b: Corresponding histology showing sheets of epidermoid cells (Haematoxylin & Eosin x 10).

cells appear like a metaplastic squamous cell. Mucin filled cells can also be demonstrated by using mucicarmine staining on cell blocks. Sometimes, epithelial cells are not aspirated and aspirate consist of only mucoid material with few histiocytes and muciphages resembling a mucocele [35]. In proper clinical contexts, such cases should be reported with differential diagnosis of low-grade MEC and must be followed-up. High-grade MEC is predominantly solid but can have a cystic component. Smears from a high-grade MEC are cellular and are readily recognisable and shows three-dimensional clusters and sheets of atypical cells with malignant squamoid features. The cells are polygonal and have a high N:C ratio with hyperchromatic nuclei often with a prominent nucleolus. If a high-grade MEC is suspected, search should be done for mucin containing vacuolated cells. Moreover, keratinsation is not the feature of MEC which can aid in distinguishing MEC from metastatic squamous cell carcinoma [35].

e. **Cystic pleomorphic adenoma (PA)** may sometimes mimic a low-grade MEC [35]. However, identification of even a focal metachromatic fibrillary material gives clues to the diagnosis. The differentiating features of PA from MEC are discussed at 4.1.1b.

f. **Papillary cystic variant of AciCC** is another important differential in cystic SG tumours [36]. It is distinguished from other low-grade carcinomas as it shows polygonal cells with fine, abundant, delicate and vacuolated cytoplasm similar to conventional AciCC but arranged in a predominant papillary pattern consisting of a cells attached to a capillary meshwork or around a fibrovascular core (**Figure 9**) [36]. The background is cystic and can show presence of lympho-histiocytes.

4.3 Tumours with clear cell and vacuolated cell pattern

The differential diagnosis of tumours of intraoral SGs comprising of clear cells and vacuolated cell pattern include – Epithelial–Myoepithelial carcinoma (EMC), Myoepithelial tumours such as myoepithelioma (ME) and myoepithelial carcinoma (MC), clear cell carcinoma (CCC), mucoepidermoid carcinoma (MEC), acinic cell carcinoma (AciCC), secretory carcinoma (SC).

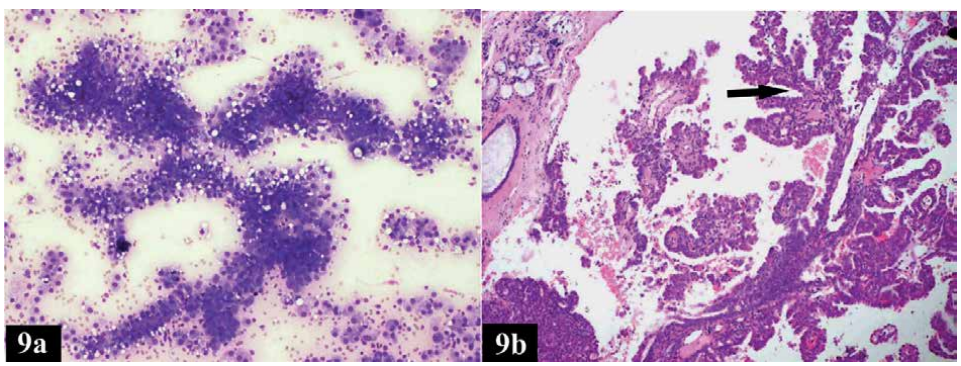

Figure 9.
9a: Showing cells arranged in a predominant papillary architecture with fine fibrovascular core in a case of acinic cell carcinoma. The cells have fine vacuolated cytoplasm (May-Grunwald Giemsa stain x 4), 9b: Corresponding histology showing branching papillae (arrow) with fibrovascular core (Haematoxylin & Eosin x 4).

4.3.1 Epithelial–myoepithelial carcinoma (EMC)

It is a an unusual tumour of major salivary gland predominantly occurring in parotid (60–80%) but can also be seen in minor SGS [20]. Palate is the most common site of occurrence and clinical presentation can be a ulcerative nodular lesion.

Key cytological features – The smears are cellular comprising of biphasic population of epithelial and myoepithelial cells. The myoepithelial cells are seen in loosly cohesive sheets, clusters and spheres with fragile, pale to clear delicate glycogen rich cytoplasm that disperses in background resulting in many naked or stripped nuclei. The nucleus of myoepithelial cells is round to oval with open chromatin and small distinct nucleoli (**Figure 10a**). Epithelial cells are seen usually in tight cohesive clusters. Sometimes mild nuclear atypia can be encountered. Hyaline stromal globules and basement membrane-like material can also be seen. The biphasic pattern of EMC can be demonstrated by using IHC on cell blocks with low molecular weight keratin and epithelial membrane antigen (EMA) for highlighting the duct cells and with smooth muscle actin (SMA), calponin, p63 and S-100 for highlighting the myoepithelial cells component [37]. Points of differentiation of EMC with other tumours with clear and vacuolated cells are discussed with each individual tumour.

4.3.2 Myoepithelioma (ME)

Myoepithelioma (ME) is a benign tumour that can occur in minor SGs with palate being the most common site. The myoepithelial cells in ME have pale to clear cytoplasm (**Figure 5b**), resembling myoepithelial cells of EMC. However, the myoepithelial cells of EMC are larger than that of ME due to presence of abundant glycogen [21]. Also, ME lacks the biphasic pattern of EMC (**Figure 10a**). ME may undergo transformation into myoepithelial carcinoma (MC). However, atypical cytological features such as nuclear pleomorphism, coarse chromatin with prominent nucleoli with background necrosis and mitotic activity seen in MC can differentiate between the two [21]. Differentiation of ME from cellular PA with predominant myoepithelial component is already discussed at 4.1.1e.

4.3.3 Clear cell carcinoma (CCC)

Clear cell carcinoma (CCC) of the SG is a rare low-grade malignancy that occurs primarily in intraoral minor salivary glands predominantly in the palate [38].

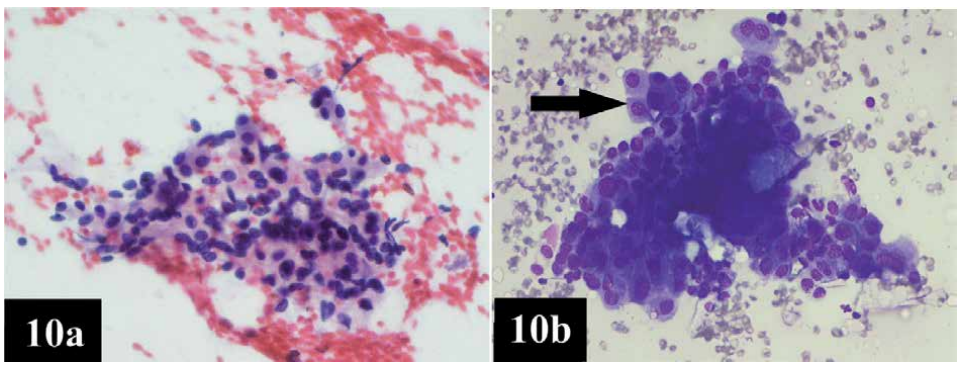

Figure 10.
10a: Smear showing admixture of dual population of cells comprising of epithelial and myoepithelial cells in a case of epithelial myoepithelial carcinoma (Haematoxylin & Eosin x 40), 10b:s Smear showing a cluster of cells with multilayering of round to polygonal oncocytic cells (arrow) with round nucleus and abundant dense granular cytoplasm in a case of an oncocytoma (May-Grunwald Giemsa stain x 40)

Epithelial cells of CCC are seen in clusters and sheets with prominent cell borders, uniform round to ovoid nuclei, granular chromatin and abundant glycogen rich clear cytoplasm**.** The cells of **CCC** may resemble myoepithelial cells of EMC. Unlike EMC, CCC lack evidence of either ductal or myoepithelial differentiation.

Clear cells can be encountered in MEC of minor SGs. However, identification of other accompanying cells (intermediate and epidermoid cells) in MEC gives clues to the diagnosis [34]. Mucin in cells of MEC can also be demonstrated by mucicarmine stain on cell blocks.

4.3.4 Acinic cell carcinoma (AciCC)

It is a low-grade tumour having predominantly vacuolated cell morphology. AciCC predominantly occurs in parotid (75–90%) and the remaining cases occur in intraoral minor salivary glands predominantly in buccal mucosa [39].

Key cytological features – Smears are cellular and consist of cells in three-dimensional clusters, sheets, micro-acinar groups and papillae with scant inconspicuous fibrovascular stroma. The cells are large with abundant fragile vacuolated basophilic to eosinophilic cytoplasm [40]. The nuclei are round, uniform with bland chromatin. Sometimes, the cells show oncocytic or clear cell changes. The background is clean with presence of bare stripped nuclei. The papillary cystic variant also has cells similar to conventional AciCC but the cells are arranged in a predominant papillary pattern with cells attached to a capillary meshwork or around a fibrovascular core (**Figure 9**). The cytoplasmic zymogen granules in AciCC can be demonstrated by PAS-D stain. Recent studies of IHC on cell blocks with DOG 1 reveals strong diffuse granular cytoplasmic positivity in AciCC [41].

Diagnostic challenges and pitfalls – Acinic Cell Carcinoma (AciCC)

a. Sometimes, aspirates from non-neoplastic SG tissue may resemble cells of AciCC. Single stripped nucleus of non-neoplastic SG may be difficult to distinguish from AciCC. However, cell groups and clusters of AciCC can be identified as they show three dimensional architecture with overlapping unlike the regularly arranged acini attached to duct with a thin fibrovascular core in a normal non-neoplastic SG tissue [40].

b. Sebaceous adenoma (SA) and sebaceous lymphadenoma are benign tumours and can occur in minor SGs and also have cells with vacuolated to clear cytoplasm [42]. Similarly, sebaceous carcinoma may arise rarely in intraoral SGs. The cells of these tumours contain cytoplasmic fat which can be demonstrated by oil-red O staining. PAS-D staining is negative for zymogen granules as seen in AciCC.

c. Cells of AciCC may show oncocyte like changes and sometimes resemble an oncocytoma**.** However, the typical cells of AciCC with finely vacuolated delicate cytoplasm can be identified. Many stripped or bare nuclei are also seen which are absent in oncocytoma [40]. Cell block preparation can be used to demonstrate periodic acid Schiff with diastase (PAS-D) positive zymogen granules in AciCC. Also, if oncocytoma is suspected, then phosphotungstic acid-haematoxylin (PTAH) stain can be done on cell blocks which shows strong positive cytoplasmic staining due to presence of abundant mitochondria.

d. AciCC may show clear cells which may resemble other tumours with clear cells such as EMC. The biphasic population of myoepithelial and epithelial cell can identified in EMC. The key cytological features of EMC are discussed at 4.3.1.

4.3.5 Secretory carcinoma (SC)

Previously called as mammary analogue secretory carcinoma, it is a new subtype of SG carcinoma and is reported to occur in minor salivary gland [43]. It also shows cells with vacuolated to clear cytoplasm.

Key cytological features – Aspirates show loosely cohesive epithelial cells arranged in small sheets, papillary fragments, acinar groups or follicular structures and also dispersed singly. The cells show variable cytoplasm ranging from granular to vacuolated and eosinophilic to clear cytoplasm. The cells show minimal or no pleomorphism. Background shows mucinous material.

Diagnostic challenges and pitfalls – The vacuolated cells may resemble cells of AciCC but lack intracytoplamic zymogen granules. Also, IHC on cell blocks is positive for markers such as mammaglobin, S-100 and vimentin and negative for DOG1 [41, 43].

4.4 Oncocytic tumours

4.4.1 Oncocytoma

Oncocytoma is a benign tumours of SG predominantly occurring in parotid. However, these tumours are also known to occur in intraoral minor SGs [44].

Key cytological features – The cells are seen in two or three dimensional and multilayered clusters. The cells are round to polygonal with oncocytic morphology consisting of dense granular abundant cytoplasm with a round nucleus and distinct nucleolus (**Figure 10b**). The background is clean without debris, fluid and lymphocytes. The oncocytic cytoplasm of cells containing abundant mitochondria can be highlighted by strong positivity for phosphotungstic acid-haematoxylin (PTAH) stain.

Diagnostic challenges and pitfalls – Oncocytic Tumours

a. The presence of oncocytes can be seen in other tumours of SGs particularly acinic cell carcinoma (AciCC) and less commonly in mucoepidermoid carcinoma (MEC) [40, 45, 46]. The distinguishing features of oncocytoma from AciCC are discussed at 4.3.5c.

b. Intraoral PA may also undergo metaplastic oncocytic change through a process known as oncocytosis. Oncocytosis is characterized by metaplastic change in SGs. Sometimes, entire salivary gland parenchyma is replaced by oncocytes mimicking an oncocytoma [45, 46]. But the typical metachromatic fibrillary matrix can usually be identified after adequate sampling in PA.

c. Oncocytic variant of mucoepidermoid carcinoma (OMEC) is a low-grade tumour and may resemble an oncocytoma as it shows bland oncocytic cells with minimal nuclear atypia [47]. However, it shows characteristic mucinous goblet cells of MEC. Also, epidermoid and intermediate cells should always be searched upon, if OMEC is suspected. Cell block can also be used to confirm mucin in goblet cells by mucicarmine staining.

d. Oncocytic carcinoma is a rare aggressive carcinoma and may develop in pre-existing oncocytoma. However, it can be distinguished from oncocytoma as it shows atypical oncocytic cells with nuclear pleomorphism. Mitosis and necrosis can also be seen.

e. Warthin's Tumour constitutes 5–15% of all salivary gland tumours. Although, it shows cohesive two or three- dimensional clusters of oncocytic cells resembling an oncocytoma but it occurs almost exclusively within the parotid gland [46]. The background is usually cystic with debris, lymphocytes and lympho-histiocytes.

4.5 Other carcinoma of intraoral salivary glands

4.5.1 Adenocarcinoma not otherwise specified (NOS)

It is an aggressive invasive tumour showing features that are not specific for any particular tumour type. It is a usually a diagnosis of exclusion. It constitutes 10–15% of all SG tumours and about 40% cases are reported in minor salivary glands with palate being the most common site [48].

Key cytological features – It shows glandular or ductal differentiation but patterns and cellular features are non-specific. The cells may be seen in nests, tubules, clusters or cords. The cellular features are variable and range from subtle low-grade tumours to a high-grade carcinoma.

Other SG carcinomas such as salivary duct carcinomas, intraductal carcinoma, lymphoepithelial carcinoma, primary squamous cell carcinoma and carcinosarcomas are rare and are reported to occur in major salivary gland and not in intraoral minor SGs.

4.6 Tumour-like lesions of intraoral salivary glands

4.6.1 Mucocele

Amongst non-neoplastic lesions, mucocele or mucous retention cyst occur in intraoral SGs and can mimic a low-grade cystic tumour. Mucocele is a pseudocyst which lack epithelial lining and contain extravasated mucin. These usually develops in minor SGs particularly on the lips and other sites such as tongue [49]. FNAC smears from mucocele are hypocellular with histiocytes and muciphages in an abundant mucoid background**.** Few giant cells can also be seen. Cystic consistency and mucoid background with muciphages may raise a possibility of low-grade MEC but other features of MEC such as intermediate and epidermoid cells are absent.

4.6.2 Sialadenosis (SA)

Sialadenosis (SA) is non-inflammatory and non-neoplastic enlargement of SGs predominantly occurring in parotid. However, it is also documented to occur in minor SGs in few reports in literature [50]. Aspirates from SA show plenty of acinar cells with hypertrophic changes. Sometimes, the cells may resemble cells of AciCC but in SA the architecture of normal SG tissue is maintained with regularly arranged acini instead of overlapping three-dimensional clusters, groups and sheets of acini in AciCC.

4.6.3 Sialadenitis

Inflammation of SGs may result from various causes but predominantly it occurs due to stenosis or obstruction of SG ducts because of sialolithiasis, trauma or secondary involvement by tumours [51, 52]. It may present with swelling and sometimes mimic a neoplasm.

4.6.4 Acute sialadenitis (AS)

Acute sialadenitis (AS) is usually a bacterial inflammation of the SGs and usually affects the parotid. However, it may also affect minor SGs [52]. Aspirates from AS usually shows abundant neutrophils, macrophages, few duct cells with reactive changes in a degenerating background. Sometimes, AS may occur as a part of underlying tumour. Non-regressive swelling on antibiotic treatment with abundant obscuring inflammation showing even focal evidence tumour such as chondromyxoid material or mucin laden or keratinized cells, should raise suspicion of a underlying hidden tumour and need to be followed-up by reaspiration or biopsy.

4.6.5 Chronic sialdenitis (CS)

Chronic sialdenitis (CS) can also affect minor SGs [53]. It may occur due to repeated episodes of inflammation or secondary to treatment such as radiation therapy for other cancers. Aspirates from CS are hypocellular and shows few clusters of duct cells, fragments of fibrous tissue in background showing lymphocytes. Acinar cells are usually not seen and are absent. The duct cells may undergo metaplastic squamous changes and may show reactive atypia, raising possibility of MEC. In such cases, other features of MEC like mucinous cells and frank epidermoid cells and dirty mucoid background give clues to the diagnosis. Frank atypical features of a carcinomatous cells such as hyperchromatic nuclei and high N:C ratio can be differentiated from reactive changes due to either chronic infection or treatment.

4.6.6 Granulomatous sialadenitis (GS)

Granulomatous sialadenitis (GS) can present clinically as a slow-growing mass that can mimic a neoplasm. Aspirates are usually hypocellular and consist of clusters of epithelioid histiocytes, multinucleated giant cells, lymphocytes and duct cells. Transforming or ill-formed epithelioid cells should not be mistaken for an epithelial neoplasm.

4.6.7 Lymphoepithelial sialadenitis (LESA)

Lymphoepithelial sialadenitis (LESA) primarily affects salivary and lacrimal glands and is also reported in minor SGS [54]. It is believed to be an autoimmune disorder and is associated with sjogrens syndrome and other connective tissue disorders. Aspirate shows lymphoepithelial complex comprising of clusters of cohesive duct cells infiltrated by lymphoid cells. The duct cells may show metaplastic squamous changes or reactive atypia. The background consist of abundant population of large and small lymphoid cells in various stages of maturation, plasma cells and tingible body macrophages. LESA is associated with increased risk of lymphoma particularly extranodal marginal zone lymphoma of MALT type and sometimes it is indistinguishable from it [55]. Ancillary tests such as IHC on cell blocks and immunophenotyping with flow cytometry should be done to distinguish between LESA and lymphoma. Reactive lymphnode can mimic LESA but lymphoepithelial complex is absent. Since LESA can be cystic, other pitfall includes cystic tumours showing background of lymphocytes such as AciCC and low-grade MEC which can be differentiated by presence of their other cytological features.

5. Conclusions

FNAC of intraoral SGs is challenging. There are many diagnostic challenges encountered in cytopathology of SG tumours as these tumours show morphological diversity and overlapping features with other neoplastic and non-neoplastic lesions. Careful assessment of morphological features with acquaintance of diagnostic challenges and pitfalls not only aid in avoiding misdiagnosis but also aid in planning further management and treatment.

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Conflict of interest

The authors declare no conflict of interest for this chapter.

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References

[1] Barnes L, Eveson J, Reichart P, Sidransky D. Tumours of the salivary glands. In: World Health Organization classification of tumors. Pathology and genetics of head and neck tumors. Lyon, France: IARC; 2005.

[2] Buchner A, Merrell PW, Carpenter WM. Relative frequency of intra-oral minor salivary gland tumors: a study of 380 cases from northern California and comparison to reports from other parts of the world. J Oral Pathol Med. 2007;36(4):207-214. doi: 10.1111/j.1600-0714.2007.00522.x.

[3] Venkata V, Irulandy P. The frequency and distribution pattern of minor salivary gland tumors in a government dental teaching hospital, Chennai, India. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111(1):e32-e39. doi: 10.1016/j.tripleo.2010.08.019. PMID: 21176809.

[4] Pires FR, Pringle GA, de Almeida OP, Chen SY. Intra-oral minor salivary gland tumors: a clinicopathological study of 546 cases. Oral Oncol. 2007;43(5): 463-470. doi: 10.1016/j.oraloncology. 2006.04.008.

[5] Ramesh M, Krishnan R, Paul G. Intraoral minor salivary gland tumours: a retrospective study from a dental and maxillofacial surgery centre in salem, Tamil Nadu. J Maxillofac Oral Surg. 2014;13(2):104-108. doi: 10.1007/ s12663-013-0489-4.

[6] Hughes JH, Volk EE, Wilbur DC. Pitfalls in salivary gland fine-needle aspiration cytology: Lessons from the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. Arch Pathol Lab Med. 2005;129:26-31. doi: 10.1043/1543-2165(2005)129<26:PISGF C>2.0.CO.

[7] Sarmento, Dmitry José de Santana et al. Minor intraoral salivary gland tumors: a clinical-pathological study. Einstein (Sao Paulo, Brazil) 2016;14(4):508-512. doi:10.1590/ S1679-45082016AO3749.

[8] Tian Z, Li L, Wang L, Hu Y, Li J. Salivary gland neoplasms in oral and maxillofacial regions: a 23-year retrospective study of 6982 cases in an eastern Chinese population. Int J Oral Maxillofac Surg. 2010 ;39(3):235-242. doi: 10.1016/j.ijom.2009.10.016.

[9] Singh M, Sagar N, Yadav S, Aggarwal R, Mandal S, Khurana N, Jain S, Meher R. Utility of Fine Needle Aspiration in Diagnosis of Intraoral Minor Salivary Gland Tumors. J Cytol. 2020;37(1):53-57. doi: 10.4103/JOC. JOC_62_19.

[10] Pal S, Mondal S, Bose K, Pradhan R, Bandyapadhyay A, Bhattacharyya D. Fine needle aspiration cytology of minor salivary gland tumors: A retrospective 5-year study of 42 cases in a tertiary care hospital. J Cancer Res Ther. 2019;15(3):686-689. doi: 10.4103/0973-1482.191055.

[11] Singh Nanda KD, Mehta A, Nanda J. Fine-needle aspiration cytology: a reliable tool in the diagnosis of salivary gland lesions. J Oral Pathol Med. 2012;41(1):106-112. doi: 10.1111/j.1600-0714.2011.01069.x.

[12] Netto Jde N, Miranda AM, da Silveira HM, dos Santos TC, Pires FR. Fine-needle aspiration biopsy as an auxiliary diagnostic tool on intraoral minor salivary gland adenoid cystic carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106(2):242-245. doi: 10.1016/j. tripleo.2008.04.015.

[13] Pusztaszeri M, Rossi ED, Baloch ZW, Faquin WC. Salivary Gland Fine Needle Aspiration and Introduction of the Milan Reporting System. Adv

Anat Pathol. 2019;26(2):84-92. doi: 10.1097/PAP.0000000000000224.

[14] Rohilla M, Singh P, Rajwanshi A, Gupta N, Srinivasan R, Dey P and Vashishta RK. Three-year cytohistological correlation of salivary gland FNA cytology at a tertiary center with the application of the Milan system for risk stratification. Cancer Cytopathology. 125:767-775. doi: 10.1002/cncy.21900.

[15] Lee SS, Cho KJ, Jang JJ, Ham EK. Differential diagnosis of adenoid cystic carcinoma from pleomorphic adenoma of the salivary gland on fine needle aspiration cytology. Acta Cytol. 1996;40(6):1246-1252. doi: 10.1159/000333988.

[16] Handa U, Dhingra N, Chopra R, Mohan H. Pleomorphic adenoma: Cytologic variations and potential diagnostic pitfalls. Diagn Cytopathol. 2009;37(1):11-15. doi: 10.1002/dc.20951.

[17] Skálová A, Andrle P, Hostička L, Michal M. Pleomorfní adenom slinných žláz: diagnostická úskalí a histologické nálezy budící podezření z malignity. Pleomorphic adenoma of salivary glands: diagnostic pitfalls and mimickers of malignancy. Cesk Patol. 2012;48(4):179-183. PMID: 23121026.

[18] Reichart PA, Kalz S, Rabel A et al. Carcinoma ex pleomorphic adenoma in a minor salivary gland: report of a case. Oral Maxillofac Surg. 2010;14:59-62. https://doi.org/10.1007/s10006-009-0183-3.

[19] Sedassari BT, Dos Santos HT, Mariano FV, da Silva Lascane NA, Altemani A, Sousa S. Carcinoma ex pleomorphic adenoma of minor salivary glands with major epithelial-myoepithelial component: clinicopathologic and immunohistochemical study of 3 cases. Ann Diagn Pathol. 2015;19(3):164-168. doi: 10.1016/j.anndiagpath.2015.03.011.

[20] Mahdavi N, Ghorbanpour M. Epithelial-Myoepithelial Carcinoma of the Palate: Report of a Case and Review of the Literatures. Iran J Pathol. 2020;15(2):144-150. doi: 10.30699/ijp.2020.105039.2076.

[21] Darvishian F and Lin O. Myoepithelial cell-rich neoplasms: Cytologic features of benign and malignant lesions. Cancer. 2004:102:355-361. doi: 10.1002/cncr.20642.

[22] Coca-Pelaz A, Rodrigo JP, Bradley PJ, Vander Poorten V, Triantafyllou A, Hunt JL, Strojan P, Rinaldo A, Haigentz M Jr, Takes RP, Mondin V, Teymoortash A, Thompson LD, Ferlito A. Adenoid cystic carcinoma of the head and neck-An update. Oral Oncol. 2015;51(7):652-661. doi: 10.1016/j.oraloncology.2015.04.005.

[23] Chang CF, Hsieh MY, Chen MK, Chou MC. Adenoid cystic carcinoma of head and neck: A retrospective clinical analysis of a single institution. Auris Nasus Larynx. 2018;45(4):831-837. doi: 10.1016/j.anl.2017.10.009.

[24] Bjørndal K, Krogdahl A, Therkildsen MH, Charabi B, Kristensen CA, Andersen E, Schytte S, Primdahl H, Johansen J, Pedersen HB, Andersen LJ, Godballe C. Salivary adenoid cystic carcinoma in Denmark 1990-2005: Outcome and independent prognostic factors including the benefit of radiotherapy. Results of the Danish Head and Neck Cancer Group (DAHANCA). Oral Oncol. 2015 Dec;51(12):1138-1142. doi: 10.1016/j.oraloncology.2015.10.002.

[25] Ben Salha I, Bhide S, Mourtzoukou D, Fisher C, Thway K. Solid Variant of Adenoid Cystic Carcinoma: Difficulties in Diagnostic Recognition. Int J Surg Pathol. 2016;24(5):419-424. doi: 10.1177/1066896916642011.

[26] Nagel H, Hotze HJ, Laskawi R, Chilla R and Droese M. Cytologic

diagnosis of adenoid cystic carcinoma of salivary glands. Diagn. Cytopathol. 1999;20:358-366. doi: 10.1002/(sici)1097-0339(199906)20:6<358::aid-dc6>3.0.co;2-x.

[27] Sodhi SP, Brar RS, Singh HP, Kaur T, Dhawan R. A rare occurrence of basal cell adenoma of palate: A case report with comprehensive immunohistochemical analysis. J Cancer Res Ther. 2015;11(4):1023. doi: 10.4103/0973-1482.147391.

[28] Werder P, Altermatt HJ, Zbären P, Bornstein MM. Canalicular adenoma of a minor salivary gland on the palate: a case presentation. Quintessence Int. 2009;40(8):623-626. PMID: 19639085.

[29] Perez-Ordonez B, Linkov I and Huvos A. Polymorphous low-grade adenocarcinoma of minor salivary glands: a study of 17 cases with emphasis on cell differentiation. Histopathology. 32:521-529. doi: 10.1046/j.1365-2559.1998.t01-2-00410.x.

[30] Gibbons D, Saboorian MH, Vuitch F, Gokaslan ST, Ashfaq R. Fine-needle aspiration findings in patients with polymorphous low grade adenocarcinoma of the salivary glands. Cancer. 1999;87(1):31-36. doi: 10.1002/(sici)1097-0142(19990225)87:1<31::aid-cncr6>3.0.co;2-g.

[31] Noonan VL, Kalmar JR, Allen CM, Gallagher GT, Kabani S. Sclerosing polycystic adenosis of minor salivary glands: report of three cases and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(4):516-520. doi: 10.1016/j.tripleo.2006.08.033.

[32] Tjioe KC, de Lima HG, Thompson LD, Lara VS, Damante JH, de Oliveira-Santos C. Papillary Cystadenoma of Minor Salivary Glands: Report of 11 Cases and Review of the English Literature. Head Neck Pathol. 2015;9(3):354-359. doi: 10.1007/s12105-014-0602-0

[33] Brannon RB, Sciubba JJ, Giulani M. Ductal papillomas of salivary gland origin: A report of 19 cases and a review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(1):68-77. doi: 10.1067/moe.2001.115978.

[34] Joseph TP, Joseph CP, Jayalakshmy PS, Poothiode U. Diagnostic challenges in cytology of mucoepidermoid carcinoma: Report of 6 cases with histopathological correlation. J Cytol. 2015;32(1):21-24. doi: 10.4103/0970-9371.155226.

[35] Pantanowitz L, Thompson LDR, Rossi ED. Diagnostic Approach to Fine Needle Aspirations of Cystic Lesions of the Salivary Gland. Head Neck Pathol. 2018;12(4):548-561. doi: 10.1007/s12105-018-0904-8.

[36] Kumar U. Acinic Cell Carcinoma Papillary-Cystic Variant: Diagnostic Pitfalls in Fine Needle Aspiration Cytology. J Clin Diagn Res. 2017;11(5):ED05-ED06. doi: 10.7860/JCDR/2017/21347.9772.

[37] Angiero F, Sozzi D, Seramondi R, Valente MG. Epithelial-myoepithelial carcinoma of the minor salivary glands: immunohistochemical and morphological features. Anticancer Res. 2009;29(11):4703-4709. PMID: 20032423.

[38] Yang S, Zhang J, Chen X, Wang L, Xie F. Clear cell carcinoma, not otherwise specified, of salivary glands: a clinicopathologic study of 4 cases and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106(5):712-720. doi: 10.1016/j.tripleo.2008.04.016.

[39] Triantafillidou K, Iordanidis F, Psomaderis K, Kalimeras E. Acinic cell carcinoma of minor salivary glands: a clinical and immunohistochemical study. J Oral Maxillofac Surg. 2010;68(10):2489-2496. doi: 10.1016/j.joms.2009.09.065

[40] Rodríguez MP, Martínez MJ, Hervás MN, Sanz AC, Vera-Sempere FJ. Cytological characteristics of acinic cell carcinoma (ACC) diagnosed by fine-needle aspiration biopsy (FNAB). A study of four cases. Oral Med Pathol. 2005;10:103-108. PMID: 15735541.

[41] Haq S, Kalakkunath S, Bakshi P, Verma K. Cytological Diagnosis of Acinic Cell Carcinoma. A Study of Four Cases with Emphasis on Differential Diagnosis and DOG1 Expression. J Cytol. 2020;37(3):155-156. doi: 10.4103/JOC.JOC_175_18.

[42] Gnepp DR, Brannon R. Sebaceous neoplasms of salivary gland origin. Report of 21 cases. Cancer. 1984; 53(10):2155-2170. doi:10.1002/1097-0142(19840515)53:10<2155::aid-cncr2820531026>3.0.co;2-f.

[43] Paudel D, Nishimura M, Adhikari BR, Hiraki D, Onishi A, Morikawa T, Neopane P, Giri S, Yoshida K, Sato J, Ono M, Kamino Y, Nagayasu H, Abiko Y. Secretory Carcinoma of Minor Salivary Gland in Buccal Mucosa: A Case Report and Review of the Literature. Case Rep Pathol. 2019;2019:2074504. doi: 10.1155/2019/2074504.

[44] Kanazawa H, Furuya T, Murano A, Yamaki M. Oncocytoma of an intraoral minor salivary gland: report of a case and review of literature. J Oral Maxillofac Surg. 2000;58(8):894-897. doi: 10.1053/joms.2000.8217.

[45] Verma K, Kapila K. Salivary gland tumors with a prominent oncocytic component. Cytologic findings and differential diagnosis of oncocytomas and Warthin's tumor on fine needle aspirates. Acta Cytol. 2003;47:221-226. doi: 10.1159/000326508.

[46] Paulino AF, Huvos AG. Oncocytic and oncocytoid tumors of the salivary glands. Semin Diagn Pathol. 1999;16(2):98-104. PMID: 10452575.

[47] Weinreb I, Seethala RR, Perez-Ordoñez B, Chetty R, Hoschar AP, Hunt JL. Oncocytic mucoepidermoid carcinoma: clinicopathologic description in a series of 12 cases. Am J Surg Pathol. 2009;33(3):409-416. doi: 10.1097/PAS.0b013e318184b36d.

[48] Li J, , Wang BY, Nelson M, Li L, Hu Y, Urken ML, Brandwein-Gensler M. Salivary adenocarcinoma, not otherwise specified: a collection of orphans. Arch Pathol Lab Med. 2004;128(12):1385-1394. Doi: 10.5858/2004-128-1385-SANOSA.

[49] Lewandowski B, Brodowski R, Pakla P, Makara A, Stopyra W, Startek B. Mucoceles of minor salivary glands in children. Own clinical observations. Dev Period Med. 2016;20(3):235-242. PMID: 27941195.

[50] Mignogna MD, Fedele S, Lo Russo L. Anorexia/bulimia-related sialadenosis of palatal minor salivary glands. J Oral Pathol Med. 2004;33(7):441-442. doi: 10.1111/j.1600-0714.2004.00208.x.

[51] Ben Lagha N, Alantar A, Samson J, Chapireau D, Maman L. Lithiasis of minor salivary glands: current data. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(3):345-348. doi: 10.1016/j.tripleo.2004.12.023.

[52] Haneke E. Acute traumatic sialadenitis of the labial and buccal minor salivary glands. Dtsch Z Mund Kiefer Gesichtschir. 1986;10(4):291-293. PMID: 3482025.

[53] Burgess LP, Quilligan JJ, Lepore ML, Yim DW. Chronic sialadenitis in a minor salivary gland. Ear Nose Throat J. 1986;65(10):485-486. PMID: 3780487.

[54] Krithika C, Sreedevi J, Sivapathasundharam B, Nithya VR. Benign lymphoepithelial lesion of the minor salivary gland - A rare

presentation as a palatal swelling. J Oral Maxillofac Pathol. 2020;24 (Suppl 1): S33-S36. doi: 10.4103/jomfp.JOMFP_17_20.

[55] Carbone A, Gloghini A, Ferlito A. Pathological features of lymphoid proliferations of the salivary glands: lymphoepithelial sialadenitis versus low-grade B-cell lymphoma of the malt type. Ann Otol Rhinol Laryngol. 2000;109(12 Pt 1):1170-1175.

Chapter 4

Personalised Precision Medicine - A Novel Approach for Oral Cancer Management

Deepa Jatti Patil and Rakesh Nagaraju

Abstract

Oral Cancer is one of the most common malignancies of the head and neck region. Despite technological advancements and improvements in Oral cancer diagnosis and treatment modalities, the 5-year survival rate remains low and is associated with poor prognosis and high mortality rate especially when detected at a later stage. The empirical therapy followed for the treatment of oral cancer includes surgery, radiotherapy and chemotherapy. The treatments are not equally efficacious for all patients, are associated with side effects and poor prognosis. The need of the hour is early diagnosis and tailored treatment therapies for individual patients. With the advent of immunotherapy, the cancer treatment has moved toward personalised precision medicine which tailors' treatments to each individual. Personalised precision medicine incorporates, molecular profiling of tumours with OMICS technology, biomarkers and companion diagnostics to build databases of patients and devise tailor made treatment approaches for individual patients. This article discusses the role of precision medicine in OSCC prevention, detection, and management by reviewing our understanding of OC from both genetic and OMICS perspectives.

Keywords: Personalised precision medicine, OMICS, Genomics, Transcriptomics, Proteomics, Metabolomics, Big data, Targetted therapy, Immunotherapy

1. Introduction

Oral cancer is a type of head and neck cancer (HNC), which encompasses a wide range of tumour types that arise from a variety of anatomic structures, including the oral cavity, oropharynx, larynx, hypopharynx, and nasopharynx. Squamous cell carcinomas (OSCCs) account for over 90% of these malignancies histopathologically, with over 50% occurring in the oral cavity [1]. Tobacco usage (smoked or chewed), arecanut, excessive alcohol use, and/or human papillomavirus (HPV) infection are the most major and well-established risk factors associated with this neoplasm [2–4].

Cancer is a major public health concern around the world. According to the International Agency for Research on Cancer's GLOBOCAN project, there were approximately 14.1 million newly diagnosed cancer cases and 8.2 million deaths worldwide in 2012.Oral cancer is one of the most common cancers worldwide, accounting for 2% of all cancer cases and having a nearly 50% mortality rate [5].

Oral and pharyngeal tumours are the sixth most common cancer worldwide [6]. Internationally, South Asian countries such as Sri Lanka, India, and Taiwan have the highest rates of oral cancer, which can be attributed to high rates of cigarette smoking and areca nut use in these countries [7].

Despite technological advancements and improvements in OSCC diagnosis and treatment modalities, the 5-year survival rate remains low, hovering around 50–60%, ranging from 80% for stage I cancers to 40% for stage IV cancers. This disparity can be explained by the delay in diagnosis as well as the relatively high tumour recurrence rates found in these patients. In general, only one-third of OSCC patients have the disease in its early stages at the time of diagnosis (I and II) [8].

Treatment strategies for OSCC differ depending on the stage of the disease at the time of diagnosis. Patients with localised disease are typically treated with surgery and/or radiotherapy, which results in a high chance of long-term survival but significant morbidity. Chemotherapy and radiotherapy and recently immunotherapy are the mainstays of treatment for metastatic OSCC [9]. Despite advances in understanding the pathobiological mechanisms of OSCC, the prognosis has not improved over the last few decades. This is largely due to the high morbidity and mortality rates associated with local and regional OSCC recurrences. The clinical challenge remains in detecting regional metastasis accurately and efficiently treating second primary OSCC and recurrent tumours [10].

The practise of medicine is still primarily empirical today, with doctors relying on pattern matching to make diagnoses based on a patient's medical history, physical examination, and test data. As a result, a prescribed treatment is frequently dependent on a physician's previous experience with patients with comparable symptoms. As a result, the best drug may be given for a "typical patient" with a certain condition. The treatment decisions are made through trial and error, and patients may experience unforeseen adverse effects or poor or no efficacy for a medicine that theoretically works in some people with that disease [11].

Traditional therapeutic procedures have a poor prognosis and are associated with negative side effects. Immunotherapy adoption has moved the field of cancer treatment toward the concept of precision and personalised medicine (PPM), which tailors' treatments to each individual. For cancer treatment, there are two options: the traditional approach and the PPM model. The fundamental distinctions between the classic cancer treatment approach and the developing precision and personalised medicine (PPM) concept are compared. Traditionally, cancer has been treated with "one-size-fits-all" treatments including chemotherapy, radiation, and surgical tumour removal. These treatments have a wide range of efficacy in different people, and they frequently destroy healthy, noncancerous organs and tissues. Individualised therapy customised to specific tissues, gene alterations, and personal characteristics relevant to each unique case of cancer characterise the PPM approach [12].

This article discusses the role of precision medicine in OC prevention, detection, and management by reviewing our understanding of OC from both genetic and OMICS perspectives.

2. Why personalised precision medicine (PPM)

Traditionally Surgery, Radiotherapy and chemotherapy have been used in the treatment of OC. Some people will only need one treatment, but most people will need a combination of medicines to combat cancer's resistance. When there are solid tumours that have not metastasized and are in easily accessible places of the

body, surgery can be utilised; nevertheless, many cancers do metastasis, necessitating more harsh therapies such as radiotherapy and chemotherapy. High doses of radiation and medicines are used in these methods to kill cancer cells and shrink tumours, but they often inflict additional damage to healthy cells [13]. It is stated that the given class of cancer medications is projected to be useless in up to 75% of patients The success of these treatments is influenced by a variety of factors, including the type, stage, and location of the cancer, as well as the patient's age and overall condition. This shows that before choosing a cancer treatment, various personal aspects should be examined [14]. Over the last decade, it has been increasingly obvious that no two patients' malignancies are exactly the same, and therefore generic treatments like chemotherapy and radiation may have varying outcomes. This standard cancer treatment strategy is extremely simple, resulting in ineffective, expensive treatments and unwanted side effects for patients [15]. It is well understood that a treatment's response varies across the variability of a population, including good and poor responders. Variables such as genetic predisposition, cohort heterogeneity, ethnicity, slow vs. quick metabolizers, epigenetic factors, and early vs. late stage of disease affect patient and therapy response. These variables influence whether or not a person will respond well to a certain treatment [11].

Immunotherapy, which uses a patient's own immune system to combat cancer, is another type of cancer treatment that has cleared the way for more specific and successful treatments. Monoclonal antibodies (mAbs), checkpoint inhibitors, cytokines, vaccinations, and adoptive cell transfer, most notably in the form of haematopoietic stem cell transplants (HSCTs) and chimeric antigen receptor (CAR) T-cell therapies, are examples of immunotherapy treatments [16]. Targeted therapeutics, such as cetuximab (monoclonal epidermal growth factor receptor [EGFR] antibody), bevacizumab (monoclonal vascular endothelial growth factor [VEGF] antibody), and mechanistic target of rapamycin (mTOR) inhibitors, have recently been introduced into treatment regimens or ongoing clinical trials to improve survival rate and reduce toxicity. With the advancement of immunotherapy, the Food and Drug Administration (FDA) has approved monoclonal antibodies that target programmed cell death protein-1 (PD-1), a receptor of the immune escape pathway, such as nivolumab and pembrolizumab, for recurrent and/or metastatic head and neck SCC [9]. Immunotherapy adoption has moved the field of cancer treatment toward the concept of personalised precision medicine (PPM), which tailors' treatments to each individual.

The purpose of PPM is to allow doctors to forecast the best course of action for a patient promptly, effectively, and precisely. Clinicians will require tools that are both compatible with their clinical workflow and cost-effective in order to achieve this. These techniques can make managing the biological complexity that underpins human diseases a lot easier. A PPM ecosystem is under constant development to enable the creation and refining of such tools, and it is the solution to the problem. Precision medicine emphasises the need of combining established clinical indicators with molecular profiling to provide diagnostic, prognostic, and therapeutic techniques tailored to the individual needs of each patient group. For the optimal utilisation of the PM ecosystem, accurate data interpretation is required. The PM ecosystem brings together omics and clinical data to solve problems [11].

A move from empirical treatment to PPM is now possible thanks to increased usage of Biomarkers and companion diagnostics (CDX) (the right medicine, for the right patient, at the right dose, at the right time) [17]. PPM is a more effective model that is ready to disrupt the "one size fits all" approach. Based on the measurement and manipulation of essential patient genetic and omic data, this perspective

promotes the creation of customised treatments for each individual subtype of cancer (transcriptomics, metabolomics, proteomics, etc.) [12].

Based on the definition provided by the National Cancer Institute, Personalised Precision Medicine, (PPM) is "an approach to patient care that allows doctors to select treatments that are most likely to help patients based on a genetic understanding of their disease."

2.1 The PPM method and its use to cancer therapy

Patients with a cancer are enrolled randomly to prevent bias in traditional drug development, employing a "all comers" method with the assumption that the enrolled patients are nearly homogeneous. The purpose of random enrollment is to guarantee that the general population is well represented. In practise, we never conduct clinical trials on patients who are randomly chosen; instead, we apply various forms of enrichments to patients' enrolment by using particular inclusion and exclusion criteria. Despite all of the efforts to enrich the community, the population that is ultimately chosen for the study can be quite diverse in terms of drug-metabolising capacity, environmental factors (e.g., nutrition, smoking habits, lifestyle, etc.), prior medication(s) exposure, and an individual's genetic and epigenetic make-up are all factors to consider. BMs are being used to better define molecular, genetic, and environmental changes. Drug developers have been studying the epigenetic makeup of patients and attempting to take a more objective stance.

Patient stratification is used to distinguish between likely responders and non-responders. When compared to randomly selected individuals, prospective stratification can result in a smaller and shorter clinical study. At a bare minimum, stratification can expedite approval for medication candidates targeted at a subset of patients while providing room for additional testing and market development in the more heterogeneous patient group. In the best-case scenario, it can reveal an effective therapeutic agent that would otherwise be lost in the noise generated by non-responders, as was the case with trastuzumab and gefitinib. This will not only decrease the duration of the clinical trial but will also be cost effective [18].

Scientists were able to read and understand an individual's genetic code, as well as detect hereditary predispositions to particular diseases, when the Human Genome Project (HGP) was completed. This watershed moment shifted the focus of health care from reactive to proactive. Scientists are currently striving to gain a detailed understanding of the body's function at numerous omics levels, as well as characterise how environmental exposures alter genetic predispositions. When all of this data is combined, scientists and doctors will be able to better anticipate how patients will respond to a particular treatment. CDx assays patients for genetic features that determine whether or not they will respond to a specific medication. This technique has the potential to have a significant impact on the patient's care. The transformation from a clinician choosing a generic medicine that is more or less experimental for the patient to one that effectively addresses the disease with PPM is the revolution [12].

2.2 Steps in PPM

1. Acquiring PPM data

2. Developing a PPM therapy

2.3 Acquiring PPM data

2.3.1 Tools for PPM

2.3.1.1 Biomarkers

Predictive BM for immunotherapy differs from typical BM used for targeted therapies in the case of cancer immunotherapy. Because of the complexity of the tumour microenvironment (TME), immune response, and molecular profiling, a more holistic approach is required than using a single analyte BM [3]. To address this issue, researchers have developed a multiplexing strategy, in which numerous BMs are used to provide more precise patient stratification. Histological analysis now includes concomitant analysis of immuno-oncology BMs, such as PD-L1 and immune cell infiltrates (**Figure 1**), as well as more comprehensive immune and tumour-related pathways (**Figure 2**) (the "Cancer Immunogram"). Multiplexed immunoprofiling, which generates a comprehensive biomarker collection that may be associated with clinical parameters, is critical for the effectiveness of PM in cancer immunotherapy [21, 22].

A specific gene or mutation must be linked to a clinical result before a PPM treatment can be created and utilised in patients. This is a significant endeavour; discovering a therapeutically relevant phenotype or polymorphism might take years of research involving many scientists. Furthermore, determining which polymorphisms cause patients to have a good or negative therapy response necessitates additional research. Sequencing DNA from a large number of people is the first step in deciphering the genetic code. This phase is becoming easier with the improvement of sequencing technologies. The most difficult issues are in interpreting these massive data sets, which is where bioinformatics comes in.

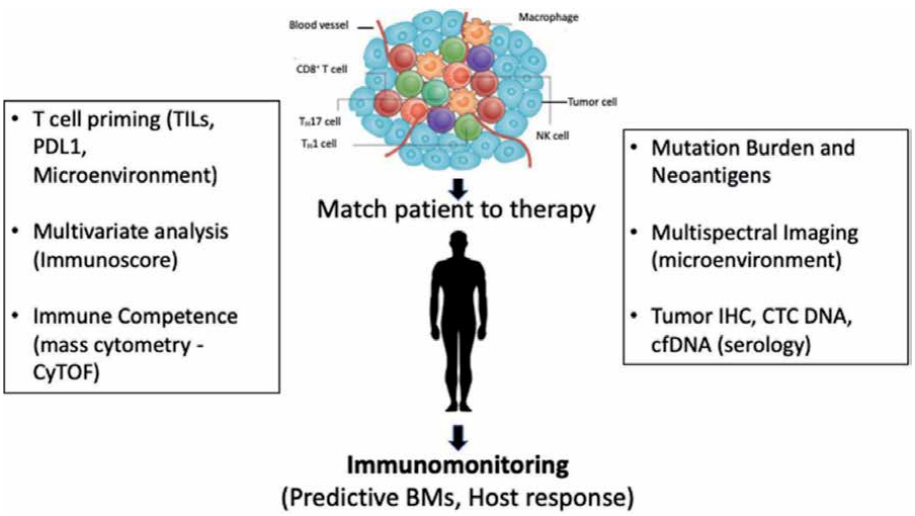

Figure 1.
Critical checkpoints for host and tumour profiling. A multiplexed biomarker approach is highly integrative and includes both tumour- and immune-related parameters assessed with both molecular and image-based methods for individualised prediction of immunotherapy response. Byassessing patient samples continuously one can collect a dynamic data on tissue-based parameters, such as immune cell infiltration and expressionof immune checkpoints, and pathology methods. These parameters are equally suited for data integration with molecular parameters. TILs: Tumour-infiltrating lymphocytes. PD-L1: Programmed cell death-ligand 1. Immunoscore: A prognostic tool for quantification of in situ immune cell infiltrates. Immunocompetence: body's ability to produce a normal immune response following exposure to an antigen (tumour drawing has been adapted from [19].

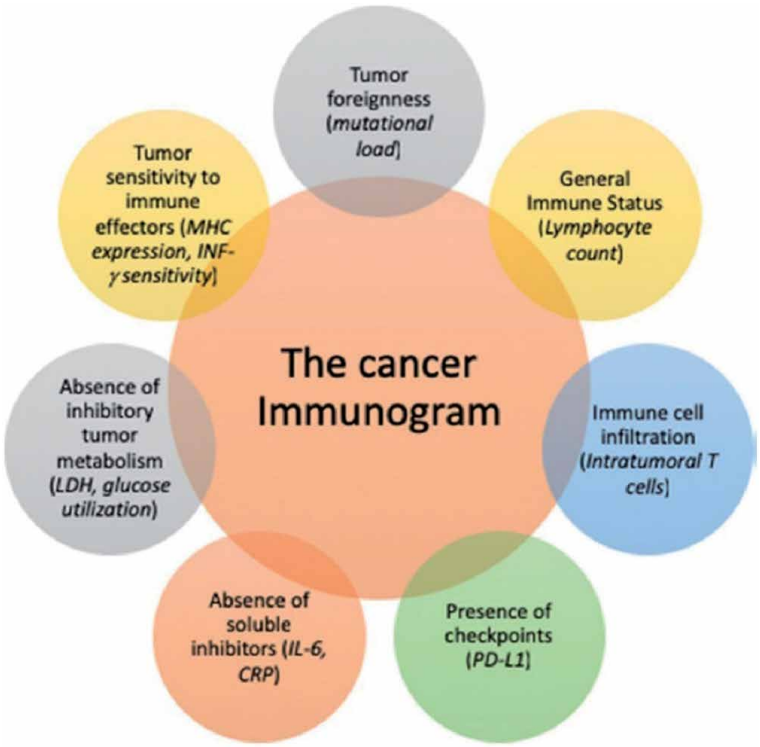

Figure 2.
The cancer immunogram. The schema depicts the seven parameters that characterise aspects of cancer-immune interactions for which biomarkers have been identified or are plausible. Italics represent those potential biomarkers for the different parameters (adapted from [20]).

Without the enormous achievement of sequencing the human genome, the discipline of PPM would not exist. From 1990 until 2003, the HGP took 13 years to complete. The International Human Genome Sequencing Consortium (IHGSC), which includes over 200 collaborators from 19 nations, was tasked with discovering new knowledge regarding the structure and organisation of the genome. The human genome has around 20,500 genes, and any two persons share 99.99 percent of their genome, implying that genetic individuality can be identified only in the remaining 0.01 percent. Long repeat sequences were also discovered in the genome, and single-base changes (single nucleotide polymorphisms [SNPs]) were found to have the potential to be distinct disease indicators. The use of bacterial artificial chromosomes (BAC) and Sanger sequencing aided in the early data collection. BAC vectors helped with the first stage of genome sequencing by determining the chromosomal location of DNA fragments recovered from a sample. Sanger sequencing, on the other hand, allowed for exact base-by-base identification of a DNA fragment. These approaches were expensive and inefficient, despite their importance in early sequencing attempts [23]. Next Generation Sequencing Technologies (NGSTs)23 have evolved as a result of years of research and development to solve these difficulties. NGSTs are a cost-effective addition to the BAC and Sanger sequencing technologies, allowing for high-dimensional and parallel sequencing [24].

Whole-genome sequencing and whole-exome sequencing are examples of genomics-related technology. There are a variety of commercial technologies for detecting gene mutations, SNPs, and copy number changes. The Cancer Genome Atlas (TCGA) is a joint project of the National Cancer Institute (NCI) and the National Human Genome Research Institute that began in 2005. In thirty-three

kinds of cancer, including head and neck SCC, the TCGA has created complete, multidimensional maps of important genetic alterations. Oral and oropharyngeal SCC has two different subgroups, according to thorough genetic profiling: HPV-negative cancers that commonly develop in the oral cavity and lips; and HPV negative cancers of the oral cavity and lips in particular. The molecular changes in these two subgroups of SCC correspond to their clinical behaviour and patient prognosis. The TCGA database demonstrated that the vast majority of HPV negative OSCCs have TP53 loss-of-function mutations and CDKN2A inactivation, which is consistent with previous findings. In addition, HPV negative OSCC showed a high amount of heterogeneity, according to integrated genomic analysis [25, 26]. Whole-exome sequencing, a transcriptomics approach for sequencing all of a genome's expressed genes, revealed new mutations that had been missed in prior studies (known as the exome. NOTCH1 mutations were found in around 15% of the patients, while mutations and focal copies of NOTCH1 were found in about 15% of the cases. NOTCH1 mutations were found in about 15% of cases, and NOTCH2/3 mutations and localised copy-number changes were found in another 11% of OSCC cases [27, 28].

OSCC's incredible diversity exemplifies how precision medicine may actually help patients and enhance medical care. The Pan Cancer Analysis of Whole Genomes project (PCAWG) is now steered to reveal noncoding driver mutations, such as alternative promoter usage, splicing, expression, editing, fusion, allele specific expression, and nonsynonymous variants, as it progresses from whole-exome sequencing to whole-genome sequencing [29]. MiRNAs and long noncoding RNAs (lncRNAs) are two types of noncoding transcripts. These noncoding transcripts, including miRNAs and long noncoding RNAs (lncRNAs), have a lot of potential for clinical research [30, 31].

2.3.1.2 Omics

While genomic data is crucial for establishing a full understanding of disease progression and therapeutic effects in physiological systems, intermediate omics levels such as the transcriptome, proteome, and metabolome are used to bridge the gap between genotypic effect and phenotypic event.

2.3.1.3 Transcriptomics

The transcriptome is the total mRNA within an individual or sample. Microarray and RNA sequencing (RNA-Seq) are two modern high-throughput sequencing approaches for collecting transcriptome data. Microarray analysis measures the amount of hybridization between a sample and corresponding probe to determine mRNA expression. The quantity of fluorescence seen within each well of the array corresponding to a given probe indicates the abundance of gene expression within a sample. Microarray analysis is constrained by the fact that designing probes requires prior knowledge of the gene's sequence. This approach is similar to Sanger sequencing in that it determines the mRNA sequence by adding fluorescently tagged nucleotide bases one by one. During each loop, fluorescent pictures are recorded, and their analysis shows the exact sequence as well as its expression level. Microarray analysis takes less time to prepare samples than RNA-Seq, although RNA-Seq does not require prior knowledge of gene sequences and may handle fewer samples. Both technologies have tremendous throughput capacities, but microarray has a higher cost-value at the moment [32, 33].

Genomic profiling enables modern medication development, which often includes either microarray analysis or RNA-Seq for transcriptome profiling. Both microarray and RNA-Seq analyses allow for the identification of disease phenotype

and medication effect within a system (single cell or bigger), which is crucial for the development of genome-specific therapeutics. Although RNA-Seq looks to be more advantageous for discovering novel genomic medication effects and disease characteristics, microarray analyses are less expensive and have more standardised techniques. In general, RNA-Seq results are better for clinical research since they have a lower signal-to-noise ratio than microarray results. Furthermore, as compared to microarray approaches, RNA-Seq results can be obtained from smaller sample quantities — nanogram versus microgram masses, respectively. As NGSTs become more widely used in clinical diagnostics, RNA-Seq methods are expected to become more standardised, eventually replacing microarray diagnostics [33, 34].

With transcriptomics technology, extensive attempts have been made to define OSCC at the molecular level. Reliable biomarkers are necessary to ease the prediction of clinical outcome and evaluate therapy efficacy in order to optimise therapeutic regimens for the management of OSCC. Dysregulation of several pathways (e.g., mRNA processing, cytoskeletal organisation, metabolic processes, cell cycle regulation, and apoptosis) was discovered when assessing a cohort of OSCC transcriptomes [35]. OSCC has also been recommended for molecular characterisation, similar to lung SCC [36]. Dysregulation of the KEAP1/ NFE2L2 oxidative stress pathway is one of the signalling pathways that has been impacted, SOX2 and TP63 lineage markers, as well as PIK3CA and EGFR mutations, were used differently. Different activation patterns of the EGFR pathway are linked to clinically diverse behaviours [37]. A molecular signature has also been proposed to help with OSCC treatment planning by predicting the existence of lymph node metastases using the primary tumour at the time of diagnosis [38]. Furthermore, microarray results demonstrated BGH3, MMP9, and PDIA3 upregulation in more than 80% of OSCC tumours, implying the relevance of ECM-cell receptor interactions in OSCC progression [39]. These transcriptional markers may be useful in the development of customised therapy regimens for the treatment of OSCC in the future.

2.3.1.4 Proteomics

The term "proteomics" refers to the process of identifying and cataloguing all proteins in a biological system, as well as their relationships. Protein structure, quantities, and cellular localizations, protein–protein interactions, and protein production and breakdown rates are all revealed by proteomic analysis. This data is utilised to figure out how the proteome changes throughout various biological activities and to spot disease patterns. Data on post-transcriptional alterations, or the quantity of proteins in a tissue, may be useful for illness diagnosis, progression, and treatment in the case of PPM. Mass spectrometry (MS) has been the primary instrument for gathering proteomic data for the past two decades, particularly to assess protein expression, identify protein modification sites, and analyse protein–protein interactions [40, 41].

The cellular abundance of proteins is primarily controlled by the quantity of translation, according to a landmark study published in 2011 that measured absolute mRNA and protein abundance and turnover using parallel metabolic pulse labelling [42]. Despite the fact that mRNA and protein levels are related to some extent, genome-wide protein abundance remains an important metric in determining cellular state and function. Intracellular and secreted proteins in body fluid specimens (e.g., serum, plasma, urine, and saliva) can be investigated using high-throughput total and phosphorylated protein analysis [43]. Alterations in protein expression in cell metabolism, adhesion, motility, and signal transduction have been discovered using proteomic analysis combined with in situ hybridization or immunohistochemistry [44, 45]. Promising results have been seen in studies.

Outcomes of salivary or serum proteomics in identifying OSCC and normal samples Analyses with a sensitivity and specificity of up to 90% [46, 47].

2.3.1.5 Metabolomics

The identification and analysis of metabolites, which are small-molecule intermediate products in metabolic reactions, is referred to as metabolomics. Because metabolites reflect both hereditary and environmental factors, a comprehensive metabolic examination is typically referred to as a "functional readout" of the current status of the organic system. The Human Metabolome Database (HMDB) is a freely accessible web resource that contains complete information on the human metabolome. The human metabolome is made up of peptides, lipids, amino acids, nucleic acids, carbohydrates, organic acids, biogenic amines, vitamins, minerals, and other tiny molecules found in the human body. The overall number of metabolites in HMDB 4.0 has increased dramatically from 40 153 in HMDB 3.0 to 114 100 in HMDB 4.0. This equates to approximately a fivefold increase [48].

In the context of PPM, metabolomic data could provide insight into an individual's unique physical reaction to a medicine, a technique known as metabolomics [49]. Currently, metabolomic studies of biofluids and tissues have aided in the development of PPM methods by discovering illness biomarkers that have the potential to aid doctors in diagnosis and early treatment. Because metabolites, unlike most proteins, travel throughout the body and appear in easily accessible biofluids like blood and urine one of the primary clinical advantages of metabolomics is that measurements may be conducted noninvasively [50]. Nuclear magnetic resonance (NMR) spectroscopy was commonly employed to identify metabolites in the early days of metabolomics research, but over the last decade, there has been a big movement toward MS, which offers superior resolution and sensitivity to small concentrations [51, 52].

Metabolite profiling of tissue and body fluid specimens with the purpose of biomarker discovery in OSCC research has revealed significant changes in energy metabolism pathways, according to mass spectrometry-based metabolomics analysis (eg, glycolysis and tricarboxylic acid cycle) [53]. Glycolytic metabolites (e.g., glucose) had higher amounts in OSCC patients' serum, while specific amino acids have lower levels (ie, valine, tyrosine, serine, and methionine) [54]. In OSCC tumour tissues, however, similar metabolite expression patterns are reversed, implying that this signature panel could be used as a screening tool. Early research, on the other hand, must be backed up with well-designed tests. Lipids have also been discovered as a significant class of metabolites, and abnormal cholesterol levels in the blood have been associated to a variety of cancers [55–57]. Total lipids, cholesterol, and high-density lipoprotein levels were shown to be considerably lower in OSCC patients compared to healthy controls [58, 59]. These lipidomic observations, albeit preliminary, may indicate greater usage of novel membrane synthesis by neoplastic cells and require further exploration.

These technologies will be collectively strong, with the potential to disclose molecular mechanisms and critical signalling pathways driving the disease, thanks to the rapid development of the omics tools outlined above. Furthermore, these tools have the potential to be utilised to identify new therapeutic targets as well as biomarkers that can be used to diagnose disease Cancer diagnosis, prognosis prediction, and treatment surveillance could all benefit from this technology.

2.3.1.6 Companion diagnostics (CDx)

The US FDA produced the first regulatory guideline document on CDx in 2014, and it was here that this type of assay was officially defined for the first time [60].

A CDx test is an in vitro diagnostic equipment that delivers information necessary for the safe and effective use of a related therapeutic product, according to this definition. This means that testing with this sort of assay is required and must be disclosed in both the medication and CDx assay labelling. CDx devices help clinicians provide the most effective, tailored medicines for their patients. In specific sections of DNA, relevant genetic information for defining malignancies can be identified (i.e., oncogenes). Some CDx are based on these specific oncogenes and can be used to evaluate whether or not a person will respond to a certain treatment in order to avoid sequencing the entire genome and obtaining superfluous information. Each CDx is linked to a certain medicinal treatment, which is linked to a particular genetic defect for which it is most effective [61]. Within the category of CDx products, there are a range of diagnostic procedures, each with its own role. Immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), and real-time quantitative PCR (RT-qPCR) are examples of these techniques [62].

2.3.1.7 Data storage

After collecting the OMICS data, storage and analysis also poses a great challenge. The International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA; sponsored by the National Cancer Institute and the National Human Genome Research Institute) are two large databases for oncology data. The data gateway of the International Cancer Genome Centre (ICGC) focuses on 50 tumour types and defines them on genomic, transcriptomic, and epigenomic levels across genders, mutations, tumour stage, and other factors. The TCGA portal has thorough information on genetic alterations and gene expression in 11 different types of cancer tissues and 33 different cancer subtypes. On a large number of patients, analysis is performed on high-quality tumour samples and matched normal tissue samples [63, 64].

2.4 Single nucleotide polymorphisms (SNPs)

Despite the lifestyle habits of exposure to high risk factors for oral cancer with 80% attributable risk of tobacco per se, a small proportion of the tobacco habit develop persistent premalignant lesions, and 3–8% transform to the malignant phenotype. Genomic variants, somatic mutations and epigenetic regulation play a critical role in oral cancer. SNPs are the most common genomic variants. SNPs are single base changes in a gene's exonic coding region or non-coding intronic regions that affect gene expression and function directly or indirectly in more than 1% of an ethnic population. SNPs in intronic regions may modify the three-dimensional structure of DNA, causing changes in molecular attributes such as Gibbs free energy aectLnJ stability, as well as impacting DNA polymerase activity and transcription factor activity. Binding SNPs can be found in one or both alleles, giving rise to heterozygous or homozygous genotypes. The wild-type (WT) allele is the ancestral allele, while the SNP allele is the changed allele. In a cancer case–control group, the frequency of allelotypes and genotypes differed, indicating a link between SNPs and cancer [65].

The connection of SNPs with risk propensity or susceptibility to oral cancer has been studied in several populations. In a meta-analysis research, the authors analysed SNPs in oral cancer and identified 34 SNPs in 30 genes that are strongly linked with oral cancer [66]. SNP rs1800471 in TGF- gene, with GC genotype associated with increased risk and GG genotype with lower risk in numerous studies and populations; SNP rs1048943 in CYP1A1 gene, with AG + GG genotypes resulting in increased risk and WT AA genotype resulting in decreased risk. The GSTM1

null genotype was linked to an elevated risk, while the WT genotype was linked to a lower risk. Similarly, heterozygous genotypes of SNPs rs1800870-AG in the IL-10 gene, rs11549467-GA in the HIF gene, and rs861539-CT in the XRCC3 genes were linked to an increased risk of oral cancer, while WT genotypes were linked to a lower risk. The WT genotypes rs1801133-CC in MTHFR and rs20417-GG in COX-2, on the other hand, were related with an increased risk, while the corresponding SNP homozygous genotypes TT and CC were associated with a decreased risk [67].

SNP analysis using high-throughput genomic analysis, as reported in genome-wide association studies (GWAS), and next-generation sequencing has emerged as a strong tool for identifying susceptibility loci, allowing information on thousands of SNPs to be obtained at the same time. These platforms often work with smaller samples and are more expensive, thus they must be confirmed in larger samples using alternative technology such as nucleotide sequencing and real-time PCR.SNPs investigated in various studies contribute to increased susceptibility to oral cancer, and a panel of SNPs could be used as Predictive Biomarkers to screen high-risk individuals who are prone to oral cancer due to tobacco use, providing an objective, unbiased test assay to assess oral cancer risk in individuals [66].

2.5 Developing a PPM therapy

2.5.1 The application of omics data to treatment

Establishing the link between biological data, disease, and clinical translation is a fundamental difficulty in PPM: how can we understand the data collected to make meaningful medical decisions? In the medical field, "Big Data" refers to a larger collection of medical data encompassing the tracking of various medical indicators and biomarkers across thousands of individuals (primarily clinical and omics data). Researchers may test tissues for thousands of molecular targets using high-throughput data gathering, effectively recording the response of a complex system over time.

The reconciliation of various omics components allows for the generation of prediction models of human physiology that may be employed in experimental design and clinical trial development in the field of systems biology [68].

Systems biologists and bioinformatic scientists use statistically significant trend detection approaches to link observations to biological events and phenotypes. Multivariate decomposition techniques, predictive modelling and optimization strategies, and other statistics-based tools are examples of these. Statistically understanding Big Data trends is a separate field that is required for predictive modelling, clinical decision-making, and assistance [69].

Drug discovery techniques for a number of PPM cancer products have been developed thanks to advances in omics technologies. Circulating tumour cells (CTC) and DNA detection approaches have promise not just for early diagnosis, but also for personalised patient risk monitoring and the development of effective personalised therapy. Several other cancer medicines in development take advantage of the immune system's particular power and specialisation to fight cancer. This has been the focus of research for almost a century, and it has evolved into a distinct discipline known as immunoengineering. The ultimate goal of this profession is to tailor a more particular and potent immune response, which can lead to a powerful and effective personalised cancer treatment [70].

2.5.2 Early cancer detection using CTCs and DNA

CTCs and circulating tumour DNA (ctDNA), two forms of oncological biomarkers, have emerged as the face of non-invasive cancer diagnosis using

"liquid biopsy" procedures. Because research has shown that tumours release both types of biomarkers into the circulation early in cancer progression, there has been a lot of focus on their use in early detection and screening. CTCs and ctDNA are likely to become more effective in risk stratification, illness monitoring, and tailored treatment selection as research progresses and technology improves [71].

230 OSCC patients at various pathological stages of the disease and treatment modes were enrolled in a cross-sectional observational study. CTCs were obtained utilising the Onco Discover liquid biopsy method, which is based on immunomagnetic CTC enumeration and has been authorised by the Drug Controller General of India. The presence of CK18 and well-defined, DAPI-stained nuclei were found in CTCs. CTC were counted and then examined for stage, extracapsular spread (ECS), lymphovascular emboli (LVE), perineural invasion (PNI), and depth of invasion, among other clinicopathological criteria (DOI). To distinguish between early and advanced stages of dialysis, CTC cut off values were obtained. CTCs in OSCC patients were found to be associated with cancer stages (clinical and pathological) and aggressive pathological characteristics. We saw a 25–50 percent increase in CTC number when aggressive clinical characteristics were present, which frequently indicate a bad prognosis. Treatment-naive patients had a reduced number of CTCs in the early stages. The number of CTCs in advanced-stage OSCC patients was 50% greater than in early-stage OSCC patients. CTC might be considered a trustworthy measure to predict the disease outcome in oral cancer due to a positive connection of CTC number with numerous pathophysiological characteristics. The presence of CTC at all stages of the disease shows that OSCC is most likely a biologically systematic disease [72].

2.5.2.1 Organoids

Patient-derived tumour organoids, which serve as in vitro tumour models and predictors of medication responses, are one strategy now under investigation for customised cancer treatment. In vitro cancer cell lines, patient-derived xenografts, and 3D culture models are all used in traditional cancer research and therapy. Due to the diversity and variability of the tumour microenvironment, these are restricted in their ability to precisely correlate an individual tumour's response to a treatment. Organoids provide a more faithful depiction of this dynamic niche, and data suggests that the genetic and functional similarities between patient-derived tumour organoids and the real thing are striking [73].

2.5.3 Targetted mononclonal antibodies for cancer therapy

Because of their low cytotoxicity, high specificity, and scalability, mAbs have proven particularly promising for cancer therapies among the numerous molecular-based approaches (e.g., small compounds, mAbs, and vaccines). mAbs are Y-shaped proteins that can attach to a specific molecular target and are created either synthetically or by B lymphocytes. mAbs are one of the most rapidly expanding immunotherapies, with over 22 FDA-approved mAb-based oncology medicines. mAb-based therapies, in contrast to standard therapies (e.g., surgery, radiation, and/or chemotherapy), are targeted to specific molecular markers expressed by a specific tumour, and so are more likely to be effective [74]. Typically, monoclonal antibodies (e.g., cetuximab) or synthetic small molecules are used to target cancer-specific cell receptors or intracellular signalling pathways (eg, gefinitib) in OC [9]. The tested drugs include, Cetuximab (Erbitux), pembrolizumab (Keytruda) and nivolumab (Opdivo).

Anti-EGFR monoclonal antibodies (mAbs) possess an antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and antitumor activity. Mice study has shown EMab-17(Anti-EGFR mAb) may be used as an antibody-based therapy for EGFR-expressing OSCC [75].

2.5.3.1 Immune check point inhibitors

The creation of antibodies capable of blocking coinhibitory immune cell receptors, or "immune checkpoints" — T-cell surface receptors that, when activated by specific ligands, limit the T-cytotoxic cell's immunological response — is a hopeful improvement in cancer treatment. Tumour cells tend to overexpress the ligands that activate these inhibitory receptors, allowing them to evade the immunological response of T cells and proliferate freely. Despite the fact that over two dozen individual costimulatory receptors have been identified, two of them — CTLA-4 and programmed cell death 1 (PD-1), have been the focus of antibody-based immune checkpoint blockade (ICB) treatments [76].

2.5.4 Non-invasive imaging for immunotherapy

Most of the patients do not respond to immunotherapy especially the immune check point inhibitors (ICI). The traditional imaging methods only provide anatomic information and do not define the concrete representation of response or progression, especially pseudo-progression due to tumour infiltrating lymphocytes (TILs); and third, toxicities are a potential concern for the widespread use of immunotherapy, which is associated with an increased risk of cancer progression. As a result, a reliable and repeatable imaging approach is critical for identifying the patient group most or least likely to react to immunotherapy [77].

Molecular imaging, in combination with disease-specific imaging probes, can provide non-invasive, early, and dynamic information about the effects of immune cells or other cells in the tumour microenvironment (TME), as well as target

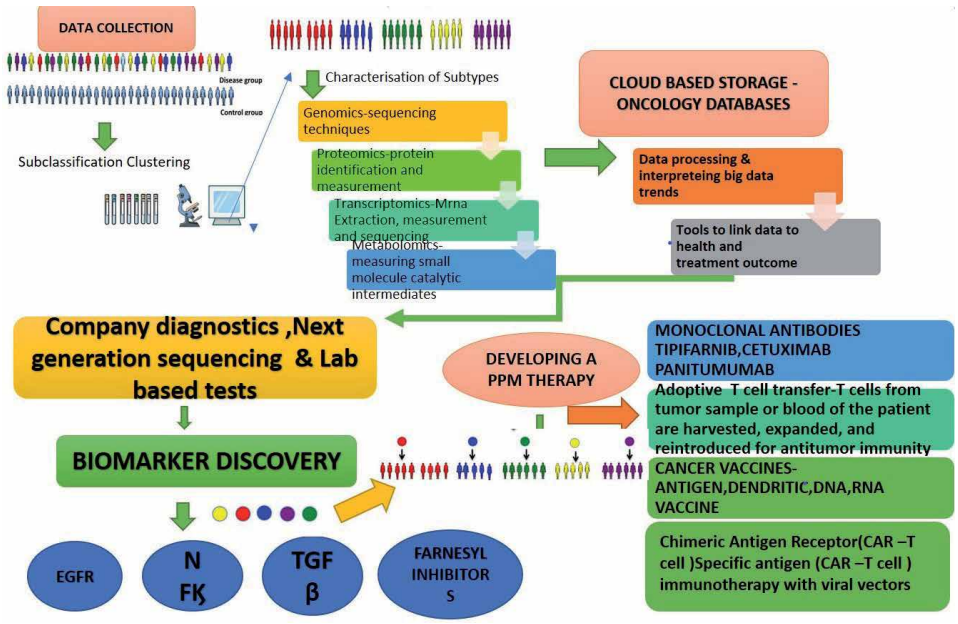

Figure 3.
Translation of PPM in to clinical practice.

expression and biodistribution of immunomodulatory drugs in the body, allowing clinicians to predict which patients will benefit most from immunotherapy. Furthermore, integrating immunotherapy with molecular imaging may improve cancer immunotherapy precision. Immunotherapies are classified into four major categories: Immune cell-based therapies, ICIs, tumour vaccines, and CAR-T cell therapy are all examples of this [78].

Figure 3 summarises the translation of PPM in to clinical practice.

3. Conclusion

This chapter has discussed the various aspects of PPM and the use of molecular and genetic profiling of tumours through omics technology for early diagnosis, formation of patient specific databases through next generation sequencing and tailored immunotherapy. Despite the advances in development technologies very few studies have been conducted in relation to OC and the research in this arena is in its budding stage. Future clinical trials on OC treatment should focus on translating the OMICs technology from bench to bedside with the use of biomarkers and CDx technologies. Tailored treatment therapies should be planned according to patients molecular and genetic profiling with consideration of individual factors. Pharma developers should create an effective medicine combining the traditional clinical data with a patient's biological profile, which includes a variety of omics-based statistics. The databases can be used to gather knowledge about disease and aid in its development more precise, safer, and better-targeted medicines for a variety of diseases in patient population.

Conflict of interest

"The authors declare no conflict of interest."

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References

[1] Forastiere A, Koch W, Trotti A, Sid D. Head and neck cancer. N Engl J Med. 2001;345(26):1890-900.

[2] Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet. 2008;371(9625):1695-1709.

[3] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev. 2005;14(2):467-475.

[4] Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, Forastiere A, Gillison ML. Improved survival of patients with human papillomavirus positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008;100(4):261-269.

[5] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136: E359–E386.

[6] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.

[7] Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life-style changes that influence survival. Oral Oncol 2010; 46:407-410.

[8] Braakhuis BJ, Brakenhoff RH, Leemans CR. Second field tumors: a new opportunity for cancer prevention? Oncologist. 2005;10(7):493-500.

[9] Algazi AP, Grandis JR. Head and neck cancer in 2016: a watershed year for improvements in treatment? Nat Rev Clin Oncol 2017; 14:76-78.

[10] Schmitz S, Ang KK, Vermorken J, et al. Targeted therapies for squamous cell carcinoma of the head and neck: current knowledge and future directions. Cancer Treat Rev 2014; 40:390-404.

[11] Seyhan, A.A., Carini, C. Are innovation and new technologies in precision medicine paving a new era in patients centric care? J Transl Med 2019; 17:114.

[12] Krzyszczyk P et al The growing role of precision and personalized medicine for cancer treatment. Technology Singap World Sci 2018; 6(3-4): 79-100.

[13] Morgan G, Ward R & Barton M The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. Clin. Oncol. (R. Coll. Radiol.) 16, 549-560 (2004). [PubMed: 15630849]

[14] Personalized Medicine Coalition. The personalized medicine report. Opportunity, challenges, and the future 2017, http://www.personalizedmedicine coalition.org/Userfiles/PMCCorporate/ file/The_PM_Report.pdf

[15] Burney IA & Lakhtakia R Precision medicine: Where have we reached and where are we headed? Sultan Qaboos Univ. Med. J 2917; 17: e255–e258.

[16] Maciejko L, Smalley M & Goldman A Cancer immunotherapy and personalized medicine: Emerging technologies and biomarker-based approaches. J. Mol. Biomark. Diagn 2017;8:

[17] Seyhan AA. Biomarkers in drug discovery and development. Eur Biopharm Rev. 2010; 1:19-25.

[18] Lee HJ, Seo AN, Kim EJ, Jang MH, Kim YJ, Kim JH et al. Prognostic and predictive values of EGFR overexpression and EGFR copy number alteration in HER2-positive breast cancer. Br J Cancer. 2014; 112:103.

[19] Nagarsheth N, Wicha MS, Zou WP. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat Rev Immunol. 2017; 17:559-572.

[20] Blank CU, Haanen JB, Ribas A, Schumacher TN. The "cancer immunogram". Science. 2016; 352: 658-660.

[21] Blank CU, Haanen JB, Ribas A, Schumacher TN. The "cancer immunogram". Science. 2016; 352:658-660.

[22] Koelzer VH, Sirinukunwattana K, Rittscher J, Mertz KD. Precision immunoprofiling by image analysis and artificial intelligence. Virchows Arch. 2018. https ://doi.org/10.1007/s0042 8-018-2485-z.

[23] Chial H. DNA sequencing technologies key to the human genome project. Nat. Educ 2008;1:

[24] Goodwin S, McPherson JD & McCombie WR Coming of age: Ten years of next-generation sequencing technologies. Nat. Rev. Genet 2016; 17:333-351.

[25] Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015; 517:576-582.

[26] Li CC, Shen Z, Bavarian R, Yang F, Bhattacharya A. Oral Cancer: Genetics and the Role of Precision Medicine. Dent Clin North Am. 2018;62(1):29-46. doi: 10.1016/j.cden.2017.08.002. PMID: 29126492.

[27] Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 2011;333:1154-1157.

[28] Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. Science 2011; 333:1157-1160.

[29] Stein LD, Knoppers BM, Campbell P, et al. Data analysis: create a cloud commons. Nature 2015; 523:149-151.

[30] Wiklund ED, Gao S, Hulf T, et al. MicroRNA alterations and associated aberrant DNA methylation patterns across multiple sample types in oral squamous cell carcinoma. PLoS One 2011;6: e27840.

[31] Tang H, Wu Z, Zhang J, et al. Salivary lncRNA as a potential marker for oral squamous cell carcinoma diagnosis. Mol Med Rep 2013;7: 761-766.

[32] Lowe R, Shirley N, Bleackley M, Dolan S & Shafee T. Transcriptomics technologies. PLOS Comput. Biol 2017;13:e1005457.

[33] Kukurba KR & Montgomery SB. RNA sequencing and analysis. Cold Spring Harb. Protoc 2015, 951-969.

[34] Buguliskis JS Could RNA-Seq become the workhorse of precision medicine? Plowing through transcriptional variations by harnessing the powerful next-gen technique. Genet. Eng. Biotechnol. News 2015; 35:8-9.

[35] Severino P, Alvares AM, Michaluart P Jr, et al. Global gene expression profiling of oral cavity cancers suggests molecular heterogeneity within anatomic subsites. BMC Res Notes 2008; 1:113.

[36] Walter V, Yin X, Wilkerson MD, et al. Molecular subtypes in head and neck cancer exhibit distinct patterns of chromosomal gain and loss of canonical cancer genes. PLoS One 2013;8: e56823.

[37] Chung CH, Parker JS, Karaca G, et al. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. Cancer Cell 2004; 5:489-500.

[38] van Hooff SR, Leusink FK, Roepman P, et al. Validation of a gene expression signature for assessment of lymph node metastasis in oral squamous cell carcinoma. J Clin Oncol 2012; 30:4104-4110.

[39] He Y, Shao F, Pi W, et al. Largescale transcriptomics analysis suggests overexpression of BGH3, MMP9 and PDIA3 in oral squamous cell carcinoma. PLoS One 2016;11: e0146530.

[40] Larance M & Lomond AI Multidimensional proteomics for cell biology. Nat. Rev. Mol. Cell. Biol 2015; 16:269-280.

[41] Han XM, Aslanian A & Yates JR Mass spectrometry for proteomics. Curr. Opin. Chem. Biol 2008;12: 483-490.

[42] Schwanhausser B, Busse D, Li N, et al. Global quantification of mammalian gene expression control. Nature 2011; 473:337-342.

[43] Malik UU, Zarina S, Pennington SR. Oral squamous cell carcinoma: key clinical questions, biomarker discovery, and the role of proteomics. Arch Oral Biol 2016; 63:53-65.

[44] Lo WY, Lai CC, Hua CH, et al. S100A8 is identified as a biomarker of HPV18- infected oral squamous cell carcinomas by suppression subtraction hybridization, clinical proteomics analysis, and immunohistochemistry staining. J Proteome Res 2007; 6:2143-2151.

[45] Hu S, Wong DT. Oral cancer proteomics. Curr Opin Mol Ther 2007; 9:467-476.

[46] Schaaij-Visser TB, Brakenhoff RH, Leemans CR, et al. Protein biomarker discovery for head and neck cancer. J Proteomics 2010; 73:1790-1803.

[47] Wadsworth JT, Somers KD, Stack BC Jr, et al. Identification of patients with head and neck cancer using serum protein profiles. Arch Otolaryngol Head Neck Surg 2004; 130:98-104.

[48] Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, *et al* HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res. 2018 4;46(D1):D608-D617.

[49] Monteiro MS, Carvalho M, Bastos ML & Guedes de Pinho P Metabolomics analysis for biomarker discovery: Advances and challenges. Curr. Med. Chem 2013;20:257-227.

[50] Roessner U & Bowne J What is metabolomics all about? Biotechniques 2009; 46:363.

[51] Everett JR, Loo RL & Pullen FS Pharmacometabonomics and personalized medicine. Ann. Clin. Biochem 2013; 50:523-545.

[52] Eckhart AD, Beebe K & Milburn M Metabolomics as a key integrator for "omic" advancement of personalized medicine and future therapies. Clin. Transl. Sci 2012; 5:285-288.

[53] Wang J, Christison TT, Misuno K, et al. Metabolomic profiling of anionic metabolites in head and neck cancer cells by capillary ion chromatography with orbitrap mass spectrometry. Anal Chem 2014; 86:5116-5124.

[54] Yonezawa K, Nishiumi S, Kitamoto-Matsuda J, et al. Serum and tissue metabolomics of head and neck cancer. Cancer Genomics Proteomics 2013;10: 233-238.

[55] Hu S, Wang J, Ji EH, et al. Targeted metabolomic analysis of head and neck cancer cells using high performance ion chromatography coupled with a Q exactive HF mass spectrometer. Anal Chem 2015; 87:6371-6379.

[56] Tie G, Yan J, Khair L, et al. Hypercholesterolemia increases

colorectal cancer incidence by reducing production of NKT and gamma delta T Cells from hematopoietic stem cells. Cancer Res 2017;77: 2351-2362.

[57] Codini M, Cataldi S, Lazzarini A, et al. Why high cholesterol levels help hematological malignancies: role of nuclear lipid microdomains. Lipids Health Dis 2016; 15:4.

[58] Acharya S, Rai P, Hallikeri K, et al. Serum lipid profile in oral squamous cell carcinoma: alterations and association with some clinicopathological parameters and tobacco use. Int J Oral Maxillofac Surg 2016; 45:713-720.

[59] Patel PS, Shah MH, Jha FP, et al. Alterations in plasma lipid profile patterns in head and neck cancer and oral precancerous conditions. Indian J Cancer 2004; 41:25-31.

[60] Papadopoulos N, Kinzler KW, Vogelstein B. The role of companion diagnostics in the development and use of mutation-targeted cancer therapies. Nat Biotechnol. 2006; 24: 985- 995.

[61] U.S. Food and Drug Administration. Mobile medical applications — Guidance for industry and food and drug administration staff (2015), https://www.fda.gov/downloads/MedicalDevices/.../UCM263366.pdf.

[62] U.S. Food and Drug Administration. List of cleared or approved companion diagnostic devices (in vitro and imaging tools) (2018), https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm.

[63] Pavlopoulou A, Spandidos DA & Michalopoulos I Human cancer databases (review). Oncol. Rep 2015;33:3-18.

[64] Kanehisa Laboratories. KEGG database (2017) http://www.genome.jp/kegg/kegg1.html.

[65] Damani Shah H, Saranath D, Pradhan S. Single nucleotide polymorphisms in transcription factor genes associated with susceptibility to oral cancer. J Cell Biochem. 2020 ;121(2):1050-1060.

[66] Multani S, Saranath D. Genotypic Distribution of Single Nucleotide Polymorphisms in Oral Cancer: Global Scene. Tumor Biol. 2016;37: 6169-6176.

[67] Multania S, Saranath D Single Nucleotide Polymorphisms and Risk of Oral Cancer: Indian Case-Control Study. J Clin Cell Immunol 2016;7: 448.

[68] Butcher EC, Berg EL & Kunkel EJ Systems biology in drug discovery. Nat. Biotechnol 2004; 22:1253-1259.

[69] McShane LM & Polley MY Development of omics-based clinical tests for prognosis and therapy selection: The challenge of achieving statistical robustness and clinical utility. Clin. Trials 2013; 10:653-665.

[70] Mackall CL Engineering a designer immunotherapy. Science 2018;359:990-991.

[71] Ried K, Eng P & Sali A. Screening for circulating tumour cells allows early detection of cancer and monitoring of treatment effectiveness: An observational study. Asian Pac. J. Cancer Prev 2017;18;2275-2285.

[72] Khandare J, Qayyumi B, Bharde A, Aland G, Jayant S, Tripathi S *et al* Correlation of CTCs with disease progression in Indian oral cancer patients. Journal of Clinical Oncology 2020 38:15 e15541-e15541.

[73] Aboulkheyr Es H, Montazeri L, Aref AR, Vosough M & Baharvand H Personalized cancer medicine: An organoid approach. Trends Biotechnol 2018;36: 358-371.

[74] Esteva FJ Monoclonal antibodies, small molecules, and vaccines in the

treatment of breast cancer. Oncologist 2004; 9:4-9.

[75] Takei J, Kaneko MK, Ohishi T, Kawada M, Harada H, Kato Y. A novel anti-EGFR monoclonal antibody (EMab-17) exerts antitumor activity against oral squamous cell carcinomas via antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Oncol Lett. 2020;19(4):2809-2816.

[76] Hargadon KM, Johnson CE & Williams CJ Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. Int. Immunopharmacol 2018; 62:29-39.

[77] Du Y, Qi Y, Jin Z, Tian J. Noninvasive imaging in cancer immunotherapy: The way to precision medicine. Cancer Lett. 2019; 466:13-22. doi: 10.1016/j.canlet.2019.08.009.

[78] R.A. Juergens, K.A. Zukotynski, A. Singnurkar, D.P. Snider, J.F. Valliant, K.Y. Gulenchyn, Imaging biomarkers in immunotherapy, Biomarkers Canc. 2016;8:1-13.

Chapter 5

Sentinel Lymph Node Biopsy for Early Oral Cavity Squamous Cell Carcinoma

Rajith Mendis and Muzib Abdul-Razak

Abstract

Early stage oral cavity squamous cell carcinoma (OCSCC) has a significant risk of subclinical nodal metastases, which is the strongest independent prognostic factor for regional recurrence and survival. However current preoperative imaging modalities are unable to identify patients with micrometastases, and an observation strategy has been associated with inferior outcomes when compared to an elective neck dissection. Sentinel lymph node biopsy provides a safe and accurate staging procedure to select the patients who benefit from an elective neck dissection, while avoiding unnecessary surgery in the patients who are node negative. There is recent Level II evidence demonstrating equivalent oncological outcomes when compared with elective neck dissection. However, a multidisciplinary approach is required including reliable mapping of the sentinel lymph node, precise surgical technique and comprehensive histopathological analysis to ensure accurate results are obtained.

Keywords: Oral squamous cell carcinoma, oral cancer, head and neck cancer, sentinel lymph node, elective neck dissection, nodal metastases, lymphoscintigraphy

1. Introduction

Early stage oral cavity squamous cell carcinoma (T1N0 or T2N0) has a significant risk of between 20 and 44% [1–3] of harbouring subclinical nodal metastases. The presence of nodal metastases has been shown to be the strongest independent prognostic factor for predicting a poor outcome [4–6]. Current imaging techniques including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and ultrasound (US) cannot accurately identify micrometastases preoperatively [7, 8]. Traditionally the only way to identify this was to perform an elective neck dissection (END), however this is unnecessary in the majority (60–80%) of patients who do not harbour occult nodal metastases, and has an associated morbidity [1]. This chapter will present the histopathological factors that have been used to risk stratify patients for an END, as well as the multifaceted technique and role of sentinel lymph node biopsy (SLNB) as a staging procedure for patients with OCSCC.

2. Histopathological factors

Various parameters have been investigated to further stratify the risk of subclinical nodal metastases, including tumour thickness and depth of invasion (DOI).

Tumour thickness measures the thickness of the tumour from the deepest point of invasion to the top of the granular cell layer, or if ulcerated, the ulcer base serves as the reference point. DOI is measured from the level of the basement membrane to the deepest point of invasion, and in the case of an ulcerated OCSCC, this level is estimated by creating an imaginary line from the adjacent basement membrane [1]. This avoids under-representing an ulcerated tumour or over-representing an exophytic tumour, and has been included in staging for OCSCC in the current 8th edition American Joint Committee on Cancer (AJCC) staging manual [9]. An increased risk of subclinical nodal metastases has been associated with varying tumour thicknesses, between 2 mm to 5 mm, with thicker tumours having a risk of nodal metastases between 44 and 50% [1, 8, 10]. The anatomical sub-site of the OCSCC may also play a role with a lesion thickness > 1.5 mm on the floor of mouth being associated with a risk of nodal metastases of 35% [11]; however, this has not been a consistent finding, with another study demonstrating a 4 mm cut-off associated with an increased risk of nodal metastases regardless of sub-site [5]. This study documented rates of local control, nodal disease, and survival rates of 91%, 8%, and 100%, respectively, for lesions <4 mm thick compared with 84%, 48%, and 74% for those ≥4 mm thick ($p < .01$). Despite this there are limitations with basing management decisions on the tumour thickness or DOI, as often this may not be assessable on a biopsy alone due to the sparse amount of biopsy material, and if assessable the biopsy may not be representative of the entire tumour [12], resulting in subsequent management decisions based more on clinical assessment.

Histopathological factors predicting for sub-clinical nodal metastases in the setting of sentinel lymph node biopsy (SLNB) have also been investigated with three variables identified including grade (G1 *vs* G2/G3), presence of lymphatic invasion and mode of invasion (cohesive *vs* dissolute) [13]. Interestingly, in this study DOI and tumour thickness were not reliable predictors of nodal metastasis demonstrating the inconsistency and uncertainty in basing management decisions on histopathological factors alone.

3. Benefit of elective neck dissection

Superior outcomes have been published in a prospective randomised controlled trial (RCT) involving patients with early OCSCC (T1/T2 tumours) without clinical evidence of nodal metastases, when they underwent an END compared to observation followed by neck dissection in the setting of nodal relapse [14]. In this study 3 year overall survival was 80% for patients undergoing END compared to 67.5% for patients undergoing delayed therapeutic dissection following relapse ($p = 0.01$). Subclinical nodal positivity in the END group was 29%, while nodal relapse rates in the observation group was 45% [14].

Of note in the 'true' node negative patients in this study, which included pathological node negative patients in the END group and those who did not relapse in the observation group, *survival was equivalent.* This demonstrates that while patients with subclinical nodal metastases benefit from a neck dissection, the remaining 60–80% of patients without nodal metastases do not experience a survival benefit by undergoing a neck dissection (see **Figure 1**). It is also important to consider that even patients with a pathologically negative neck following END have a rate of regional failure up to 5–10% [3, 15].

This benefit of END has been reported in a previous observational study where patients with early (T1/T2) OCSCC had significantly improved outcomes undergoing END (median survival 12 years) compared to observation (median survival 4.1 years), with the majority (11/12) of recurrences in the observation group

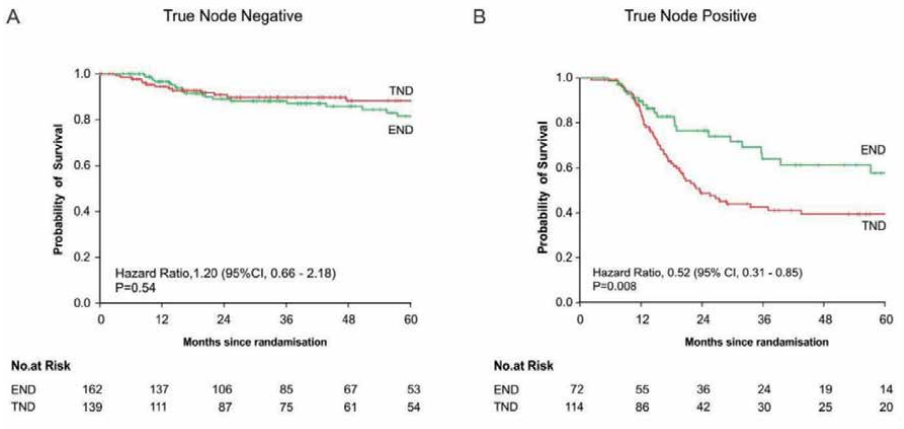

Figure 1.
Overall survival in 'true node negative' and 'true node positive' patients [14].

occurring as regional failures [2]. This benefit is selectively achieved in patients undergoing SLNB, as patients with a positive sentinel lymph node (SLN) undergo a completion neck dissection while the morbidity of the neck dissection is avoided when the SLNB is negative.

4. Technique of sentinel lymph node biopsy

There is marked heterogeneity in the published data assessing the role of SLNB in OCSCC including preoperative investigations, technique of identifying the SLN and the pathological assessment of the specimens [16]. The GETTEC (Groupe d'Etude des Tumeurs de la Tête et du Cou) guidelines [17] have attempted to standardise the technique in performing SLNB with recommendations for lymphoscintigraphy, surgery and pathological analysis. Of note, they recommend a median of three SLNs to be sampled, with a single SLN node considered insufficient to accurately determine the nodal pathological status.

SLNB for OCSCC presents unique challenges in relation to both the complex anatomy of the head and neck, in addition to the short distance between the primary lesion and the draining nodal basin, particularly for lesions located in the floor of mouth. This is due to the high activity at the adjacent injection site, which can be easily overlooked by planar lymphoscintigraphy and intraoperative gamma probes [18]. Intraoperatively, the close relationship between the primary lesion and the draining lymph nodes can result in so called 'shine through' of the radioactive tracer from the primary site with difficulties in identifying the SLN if it is in an adjacent nodal basin, particularly the submental (IA) and submandibular (IB) basins. Composite single photon emission computed tomography (SPECT) with concurrent CT combines functional and anatomical imaging to enhance topographic orientation and diagnostic sensitivity, with more SLNs being detectable than by planar lymphoscintigraphy alone, as well as providing more detailed anatomical information to assist with intraoperative localisation [19]. **Figures 2** and **3** demonstrates the lymphoscintigraphy result and composite SPECT/CT for patients with unilateral and bilateral lymphatic drainage respectively. The SPECT/CT provides detailed anatomical information to assist with identification of the SLN.

Another consideration that may impact on the accuracy of lymphoscintigraphy is the choice of radiotracer. These have different molecular characteristics as

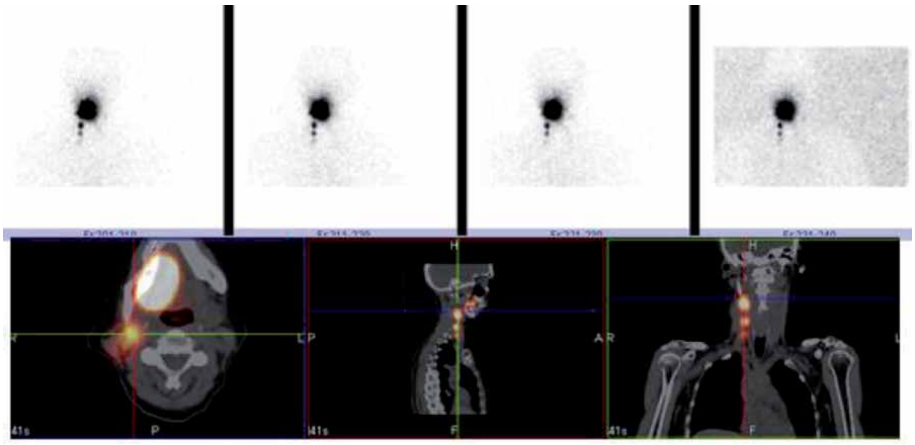

Figure 2.
Lymphoscintigraphy and SPECT/CT demonstrating ipsilateral level 2 sentinel lymph node.

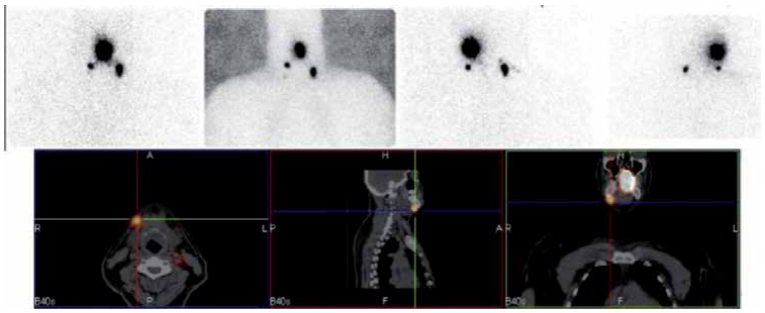

Figure 3.
Lymphoscintigraphy and SPECT/CT demonstrating bilateral drainage from a lateralised tumour.

summarised in **Table 1**, which impacts the drainage characteristics, and this may be utilised to counteract the 'shine through' effect. The potential of [^{99m}Tc]Tilmanocept is of particular interest as it has a small molecular size of 7 nm facilitating rapid injection site clearance, and targets the CD206 receptor found on the reticuloendothelial cells in lymph nodes to promote accumulation within the SLN while reducing drainage to second tier nodes [21, 22]. A study assessing [^{99m}Tc]Tilmanocept in the setting of both OSCC and head and neck cutaneous SCC demonstrated a SLN detection rate of 97.6%, with a false negative rate of 2.56% [22]. This study included

Agent	Mean Particle size (nm)
Sulphur Colloid	100–220
Antimony trisulphide	3–30
Suphide nanocolloid	10–50
Nanocolloidal albumin	5–80
Rhenium sulphide nanocolloid	50–200
ICG-99mTc-Nanocolloid	5–80
Tilmanocept	~7

Table 1.
^{99m}TC labelled radiotracers [20].

20 patients with floor of mouth OSCCs, where [^{99m}Tc]Tilmanocept may be of particular use, and a SLN was successfully identified in all cases without any false negatives [22]. A recent comparison study between [^{99m}Tc]Tilmanocept and [^{99m}Tc] Nanocolloid found that [^{99m}Tc]Tilmanocept had higher rates of clearance from the primary injection site but also had reduced accumulation within the SLN, with a similar SLN to injection site ratio of radioactivity between the two radiotracers [23].

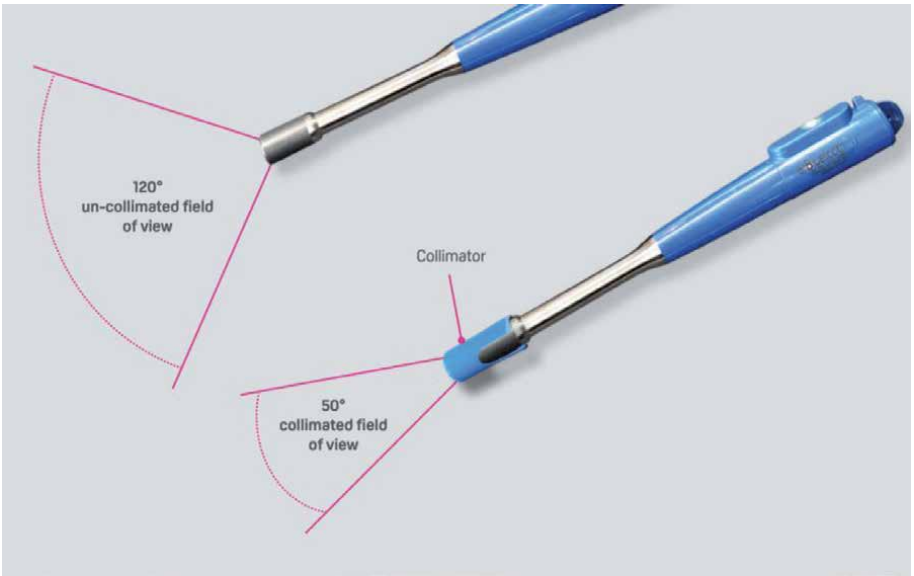

Figure 4.
Focused field of view with use of collimator. (This image is © 2021 Devicor Medical Products, Inc.; used with permission).

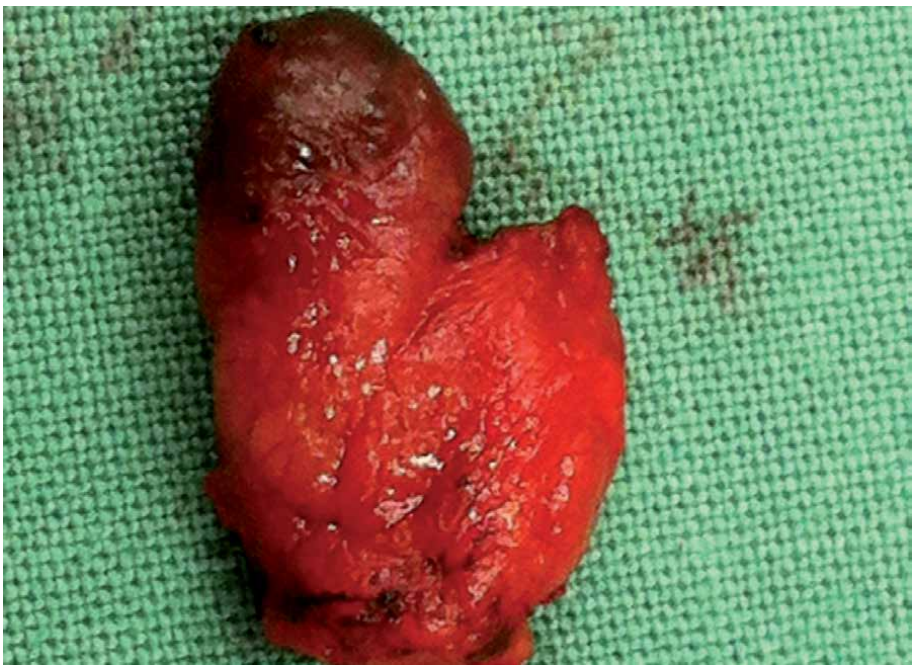

Figure 5.
Sentinel lymph node with blue dye on one surface facilitating visual identification.

This study demonstrated a high degree of agreement in the identification of SLNs between each radiotracer, however it is difficult to draw definitive conclusions with a small sample size, and further studies are required.

Specific surgical techniques can be employed to counteract the 'shine through' effect, including mobilisation of the fat pad between the submandibular gland and anterior belly of digastric to reflect the tissue, allowing for careful analysis with the handheld gamma probe while avoiding radiation from the primary tumour injection site [24]. A probe with an angled head (Neoprobe, Devicor Medical Products) with collimator attached is indispensable in such narrow spaces as in the neck to reliably locate the node. The collimator serves to decrease the field of view from 120 to 50 degrees while simultaneously increasing the spatial resolution of the probe (see **Figure 4**). Selective use of patent blue dye (Aspen Pharmacare) when the draining lymph nodes are in the submental and submandibular basins provides additional visual information to assist with identification of the SLN as demonstrated in **Figure 5**.

5. Histopathological analysis

The presence of nodal micrometastases (0.2 mm–2 mm) or isolated tumour cells (ITC) (<0.2 mm) [9] may be overlooked by standard histopathological analysis, with one study that reanalysed 76 neck dissection specimens with serial sectioning identifying previously undetected micrometastases in 7.9% of specimens [25]. These metastases occurred mainly in small (<1 cm) lymph nodes, without extranodal extension and therefore would not have been routinely identified on preoperative imaging. **Figure 6** demonstrates how micrometastases are detected more reliably by performing serial sectioning. Another smaller prospective study analysed 34 neck dissection specimens with serial sectioning and immunohistochemistry (IHC) in addition to standard haematoxylin–eosin staining (HES) and found that 3 patients (8.8%) were upstaged by the additional analysis, with two cases of micrometastases and one patient harbouring ITC [26]. Importantly the identification of these micrometastases did not warrant further treatment beyond the neck dissection which had already been performed [25]. However, the revised findings of node positivity has both a staging and prognostic impact on patients.

In another study in the setting of SLNB, serial sectioning and IHC upstaged 5 of 27 (19%) patients with nodal metastases [8], and a retrospective review of 272

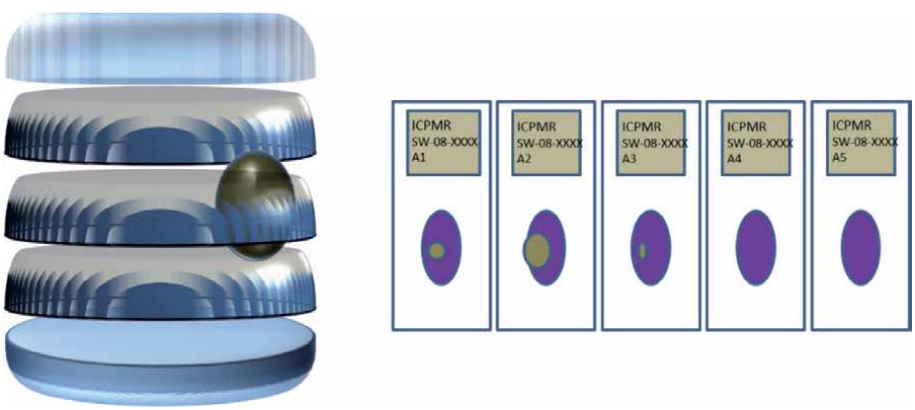

Figure 6.
Serial sectioning a lymph node.

patients undergoing SLNB found that 51.7% of their positive sentinel lymph nodes were only detected following serial sectioning and IHC [27]. The addition of IHC to standard HES increases both the sensitivity and negative predictive value of SLNB [16, 28, 29] and has now become part of the standard pathological assessment of SLNs in most institutions.

Performing serial sectioning and IHC (cytokeratin – AE1/AE3) is both labour and time intensive for the pathologist. By performing a SLNB, the detailed examination can be focused on the most likely lymph nodes which might harbour micrometastatic disease for each individual patient, providing the most precise staging and prognostic information.

6. Accuracy in predicting neck status

The Sentinel European Node Trial (SENT) was a large multicentre European study investigating SLNB in 415 patients with early OCSCC, of which subclinical nodal metastases were identified in 26% of the study population. The findings demonstrated the procedure to be safe, reliable and accurate with a SLN identified in 99% of cases, with 86% sensitivity, 95% negative predictive value and 14% false negative rate [30]. These results have been replicated in other similar studies, albeit with lower false negative rates of 2.56% [22] and 9.1% [31], and with higher rates of contralateral drainage (23–40%) [31, 32].

SLNB allows for identification of unexpected lymphatic drainage patterns, and the SENT trial found that bilateral drainage was identified in 10% of lateralised tumours, and 2.4% had exclusive contralateral drainage. The patients with contralateral drainage, 7 of 49 had positive SLNs, with 5 of the patients draining exclusively contralaterally [30]. The rate of contralateral drainage for lateralised tumours has been documented in other studies to be as high as 23–40% [31, 32].

The detection of contralateral drainage is a major benefit of performing a SLNB as it allows accurate mapping of the lymphatic drainage for each individual patient, and for patients with lateralised tumours with contralateral drainage, these nodes will not be addressed if they undergo a unilateral END. If there were undetected subclinical nodal metastases in these nodes, these patients would then be at risk of a contralateral nodal failure.

The accuracy of SLNB has been further investigated by a systematic review/meta-analysis assessing the performance of SLNB as a staging procedure for OCSCC and documented it to be reliable with a sensitivity of 88% and specificity of 99%. However, when assessing covariates, performing IHC on the SLN significantly improved the sensitivity to 93% [29]. In addition to the differences in processing of specimens, there was a degree of heterogeneity in the articles in relation to measurement of failure with a combination of END and clinical follow up to detect potential false negatives. Despite this the review demonstrated that SLNB is highly accurate across several different institutions, with an improvement in quality of life including pain, shoulder mobility and scarring when compared to END [29].

7. Outcomes following sentinel lymph node biopsy

A systematic review assessing outcomes in patients with early OCSCC managed with either a SLNB or END found no significant difference in overall survival or disease free survival between the two approaches [33]. This study analysed 5 separate studies with a total of 560 patients and reported 10 more neck recurrences per 1000 patients undergoing the SLNB strategy compared with END, although this

was not statistically significant. Conversely SLNB avoided the need for a neck dissection in 64% of patients. While this did demonstrate robust outcomes for patients treated with SLNB, none of the included studies were randomised and as such the overall quality of the evidence was considered low.

Two RCTs have been subsequently published comparing SLNB and END for early OCSCC with both demonstrating equivalent oncological outcomes, and their findings are summarised in **Table 2** and **Figure 7**. The Senti-MERORL trial was a multi-centre RCT with 307 patients that documented a 25% rate of SLN positivity, with these patients proceeding to a neck dissection [15]. There was a mean follow up of 4.95 years, and rates of nodal recurrence were 10.1% in the neck dissection group and 9.3% in the SLNB group, which was not a statistically significant difference. Equivalent locoregional disease control, disease specific survival and overall survival were demonstrated at 2 and 5 years [15]. When looking at the nodal recurrences in patients initially classified as pathologically node negative (pN0), there were 11 patients (10% of the 109 pN0 patients) in the END group and 8 patients (8% of the 99 pN0 patients) in the SLNB group, demonstrating similar rates of nodal staging failure between the two strategies.

A Japanese RCT compared 137 patients in the neck dissection arm and 134 patients in the SLN arm. They found a 34% rate of SLN positivity, and regional recurrence rates were 9.5% and 11.2% in the END and SLNB groups respectively.

	Garrel et al. [15]		**Hasegawa et al. [34]**	
	SLNB (n = 140)	**END (n = 139)**	**SLNB (n = 134)**	**END (n = 137)**
Age	60.8 (mean)	59.1 (mean)	63 (median)	63 (median)
Sex				
Male Female	88 (63%) 52 (37%)	101 (73%) 38 (27%)	89 (66%) 45 (34%)	90 (66%) 47 (34%)
Site of primary				
Oral Tongue	124 (89.2%)	119 (85.6%)	109 (81.3%)	114 (83.2%)
Floor of mouth			13 (9.7%)	14 (10.2%)
Lower gingiva			7 (5.2%)	6 (4.4%)
Buccal mucosa			5 (3.7%)	3 (2.2%)
Oropharynx	15 (10.8%)	20 (14.4%)		
T1	88 (63%)	91 (66%)	26 (19%)	25 (18%)
T2	52 (37%)	48 (35%)	108 (81%)	112 (82%)
Positive lymph nodes	35 (25%)[a]	30 (22%)	46 (34%)	34 (25%)
Frozen section/ Imprint cytology	21 (15%)		32 (24%)	
H&E/IHC	12 (9%)		14 (10%)	
Adjuvant treatment				
Radiotherapy Chemoradiotherapy	23 (16%) 10 (7%)	28 (20%) 6 (4%)	4 (3%) 0	3 (2%) 3 (2%)
Nodal recurrence	13 (9%)	14 (10%)	15 (11%)	13 (10%)
Follow up (months)	56.9 (mean)	59.4 (mean)	37 (median)	37 (median)
Overall Survival	82.2% at 5 y	81.8% at 5 y	87.9% at 3 y	86.6% at 3 ys

[a]*Includes 2 positive cases out of 8 undergoing END due to localization failure.*

Table 2.
Comparison of two RCTs.

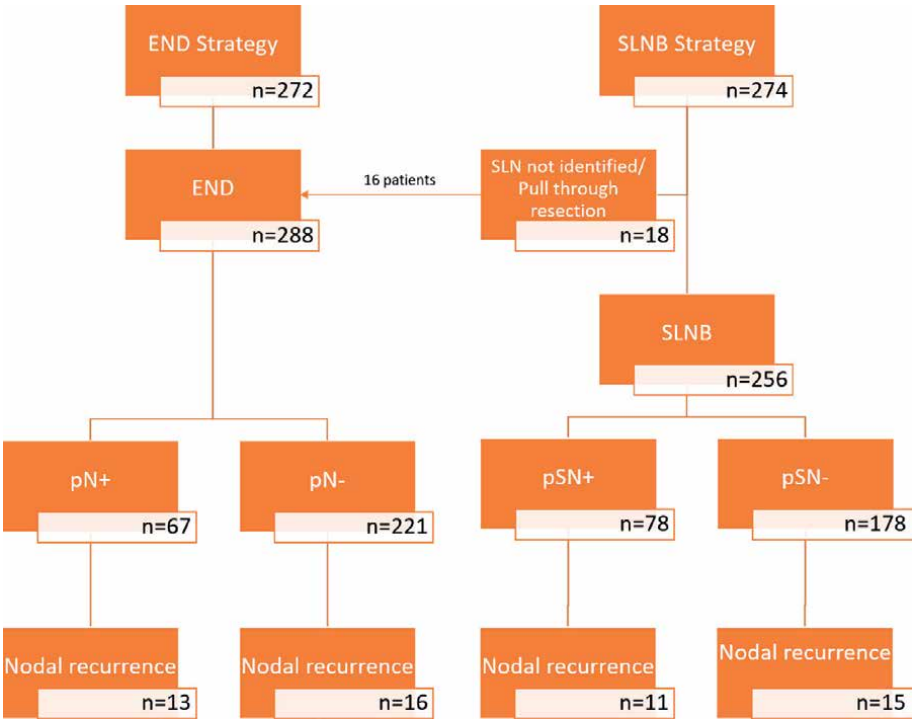

Figure 7.
Combined RCT outcomes of nodal recurrence. (Adapted from Garrel et al. [15], Hasegawa et al. [34]).

This study demonstrated equivalent 3 year overall survival and disease free survival between the END group (87.9% and 81.3%) and the SLN group (86.6% and 78.7%) [34]. Both studies demonstrate high-level evidence to support the use of SLNB as a staging procedure for patients with early T1 or T2 OCSCC.

END has an associated morbidity including shoulder dysfunction, pain and contour changes [16]. Comparison of morbidity associated with SLNB or a neck dissection demonstrates low rates of morbidity overall, however, in one study all the morbidity occurred following neck dissection, with no cases of shoulder dysfunction in the SLNB group [6]. Quality of life assessments demonstrate improved tactile sensitivity and reduced pain sensitivity in the SLNB group, with no significant difference in the presence of lymphoedema although there was trend towards improved symptoms in the sentinel lymph node biopsy group [35]. Functional outcomes were also assessed in the two RCTs, with the Senti-MERORL study finding an initial functional difference between the two groups favouring SLNB at 6 months, however this resolved by 12 months [15]. Hasegawa reported that the END group had persisting inferior scores at 12 months post operatively, when assessing neck stiffness and shoulder dysfunction compared to the SLN group [34].

8. Future directions

It is widely accepted that macrometastases and micrometastases should undergo a completion neck dissection, however management of ITC remains uncertain without a clear consensus. This is a significant issue as the incidence of ITC ranges between 14 and 27% of positive SLNs [27, 30, 36] and the two RCTs managed this subgroup with differing strategies. The Senti-MERORL trial treated ITC with observation, and in those 11 patients there were no nodal recurrences [15]. Conversely

the Japanese RCT treated ITC with a completion neck dissection [34], however subgroup outcome data was not published. A retrospective Dutch study analysing outcomes for patients undergoing SLNB for OCSCC found a SLN positivity rate of 22% (107/488 patients) and of these patients, 15 (14%) had ITC, 31 (29%) had micrometastases and 61 (57%) had micrometastases. 13 of the patients with ITC underwent a neck dissection with 1 patient having additional positive lymph nodes, and the other 2 patients had adjuvant radiotherapy, and did not develop regional recurrence during follow up [36]. While ITC is considered to represent node negative disease in the setting of breast cancer [9], management of these patients remain uncertain in the setting of OCSCC and further data is required to clarify both the natural history and management outcomes for this subset of patients.

Intraoperative lymphoscintigraphy is a developing technique which has particular utility in the management of oropharyngeal or laryngeal SCC with a SLNB. These tumours are unable to be injected with a radiotracer in an awake patient for a preoperative assessment [37]. Indocyanine green (ICG) is readily taken up by lymphatics and can be identified intraoperatively using a near-infrared fluorescence camera to locate the sentinel lymph node [38]. The use of ICG does not cause any staining of the primary site as seen with use of patent blue dye, and also provides an immediate result, which offers obvious benefits in the setting of intraoperative sentinel lymph node identification. However, it does not provide the detailed drainage information with anatomical referencing that is provided by performing radiotracer based lymphoscintigraphy with a SPECT/CT. While techniques such as skin compression have been described to identify lymphatic drainage and the SLN before making a skin incision [37], often the skin flaps need to be raised to comprehensively assess the nodal basins [39]. In addition, the ICG signal spreads rapidly with time and thus second tier lymph nodes can be hard to distinguish from the true sentinel lymph node [40]. The use of hybrid tracers which assemble ICG with a radiocolloid to increase the retention time in the sentinel lymph node has been described [38], and may have an increasing future role, along with the use of intraoperative SPECT scanners, to counteract the disadvantages of using ICG alone. However, this is an exciting new tool which can be utilised to expand the utility of the SLN technique.

9. Conclusion

Early OCSCCs have a risk of subclinical nodal metastases to the draining cervical lymph nodes, which has a negative impact on the patient's prognosis and survival. The subclinical nature limits the ability to identify these with current imaging techniques including a PET scan. Despite this, there is recent high quality evidence demonstrating that treating this disease surgically has superior survival outcomes compared with an observation strategy. However, the patients *without* subclinical nodal metastases (up to 80%) do not gain any benefit by undergoing a neck dissection.

SLNB technique represents a minimally invasive technique allowing treatment de-intensification without compromising the oncological efficacy. SLNB has been demonstrated to provide an accurate and safe staging procedure to assess for subclinical nodal metastases with added benefits over an END including identification of out-of-field drainage, as well as a more detailed pathological assessment of the SLN However, a high quality multidisciplinary approach is required including accurate preoperative lymphoscintigraphy, precise surgical technique and detailed pathological assessment to ensure reliable results and good patient outcomes.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Pentenero M, Gandolfo S, Carrozzo M. Importance of tumor thickness and depth of invasion in nodal involvement and prognosis of oral squamous cell carcinoma: A review of the literature. Head Neck 2005; 27: 1080-1091.

[2] Ebrahimi A, Ashford BG, Clark JR. Improved survival with elective neck dissection in thick early-stage oral squamous cell carcinoma. Head Neck 2012; 34: 709-716.

[3] Ferris RL, Kraus DH. Sentinel lymph node biopsy versus selective neck dissection for detection of metastatic oral squamous cell carcinoma. Clin Exp Metastasis 2012; 29: 693-698.

[4] Gurney BAS, Schilling C, Putcha V, et al. Implications of a positive sentinel node in oral squamous cell carcinoma. Head Neck 2012; 34: 1580-1585.

[5] O'Brien CJ, Lauer CS, Fredricks S, et al. Tumor thickness influences prognosis of T1 and T2 oral cavity cancer - But what thickness? Head Neck 2003; 25: 937-945.

[6] Murer K, Huber GF, Haile SR, et al. Comparison of morbidity between sentinel node biopsy and elective neck dissection for treatment of the n0 neck in patients with oral squamous cell carcinoma. Head Neck 2011; 33: 1260-1264.

[7] Stoeckli SJ, Haerle SK, Strobel K, et al. Initial staging of the neck in head and neck squamous cell carcinoma: A comparison of CT, PET/CT, and ultrasound-guided fine-needle aspiration cytology. Head Neck 2012; 34: 469-476.

[8] Ross GL, Soutar DS, MacDonald DG, et al. Improved staging of cervical metastases in clinically node-negative patients with head and neck squamous cell carcinoma. Ann Surg Oncol 2004; 11: 213-218.

[9] Amin MB, Edge S, Greene F, et al. *AJCC Cancer Staging Manual*. 8th ed. 2017.

[10] Yuen APW, Lam KY, Lam LK, et al. Prognostic factors of clinically stage I and II oral tongue carcinoma - A comparative study of stage, thickness, shape, growth pattern, invasive front malignancy grading, Martinez-Gimeno score, and pathologic features. Head Neck 2002; 24: 513-520.

[11] Mohit-Tabatabai MA, Sobel HJ, Rush BF, et al. Relation of thickness of floor of mouth stage I and II cancers to regional metastasis. Am J Surg 1986; 152: 351-353.

[12] Bundgaard T, Bentzen SM, Wildt J. and Clinical Parameters in the Prognostic Evaluation of Squamous Cell Carcinoma of. 1996; 142-152.

[13] Goerkem M, Braun J, Stoeckli SJ. Evaluation of clinical and histomorphological parameters as potential predictors of occult metastases in sentinel lymph nodes of early squamous cell carcinoma of the oral cavity. Ann Surg Oncol 2010; 17: 527-535.

[14] D'Cruz AK, Vaish R, Kapre N, et al. Elective versus Therapeutic Neck Dissection in Node-Negative Oral Cancer. N Engl J Med 2015; 373: 521-529.

[15] Garrel R, Poissonnet G, Plana AM, et al. Equivalence randomized trial to compare treatment on the basis of sentinel node biopsy versus neck node dissection in operable T1-T2N0 oral and oropharyngeal cancer. J Clin Oncol 2020; 38: 4010-4018.

[16] Yang Y, Zhou J, Wu H. Diagnostic value of sentinel lymph node biopsy for cT1/T2N0 tongue squamous cell carcinoma: a meta-analysis. Eur Arch Oto-Rhino-Laryngology 2017; 274: 3843-3852.

[17] Garrel R, Poissonnet G, Temam S, et al. Review of sentinel node procedure in cN0 head and neck squamous cell carcinomas. Guidelines from the French evaluation cooperative subgroup of GETTEC. Eur Ann Otorhinolaryngol Head Neck Dis 2017; 134: 89-93.

[18] Wagner T, Buscombe J, Gnanasegaran G, et al. SPECT/CT in sentinel node imaging. Nucl Med Commun 2013; 34: 191-202.

[19] Meerwein CM, Sekine T, Veit-Haibach P, et al. Multi-slice SPECT/CT vs. lymphoscintigraphy and intra-operative gamma ray probe for sentinel node mapping in HNSCC. Eur Arch Oto-Rhino-Laryngology 2017; 274: 1633-1642.

[20] Giammarile F, Schilling C, Gnanasegaran G, et al. The EANM practical guidelines for sentinel lymph node localisation in oral cavity squamous cell carcinoma. Eur J Nucl Med Mol Imaging 2019; 46: 623-637.

[21] Wallace AM, Hoh CK, Vera DR, et al. Lymphoseek: A molecular radio-pharmaceutical for sentinel node detection. Ann Surg Oncol 2003; 10: 531-538.

[22] Agrawal A, Civantos FJ, Brumund KT, et al. [99mTc] Tilmanocept Accurately Detects Sentinel Lymph Nodes and Predicts Node Pathology Status in Patients with Oral Squamous Cell Carcinoma of the Head and Neck: Results of a Phase III Multi-institutional Trial. Ann Surg Oncol 2015; 22: 3708-3715.

[23] den Toom IJ, Mahieu R, van Rooij R, et al. Sentinel lymph node detection in oral cancer: a within-patient comparison between [99mTc]Tc-tilmanocept and [99mTc]Tc-nanocolloid. Eur J Nucl Med Mol Imaging 2021; 48: 851-858.

[24] Stoeckli SJ, Huebner T, Huber GF, et al. Technique for reliable sentinel node biopsy in squamous cell carcinomas of the floor of mouth. Head Neck 2016; 38: 1367-1372.

[25] Ambrosch P, Kron M, Fischer G, et al. Micrometastases in carcinoma of the upper aerodigestive tract: Detection, risk of metastasizing, and prognostic value of depth of invasion. Head Neck 1995; 17: 473-479.

[26] Majumdar KS, Rao VUS, Prasad R, et al. Incidence of Micrometastasis and Isolated Tumour Cells in Clinico-pathologically Node-Negative Head and Neck Squamous Cell Carcinoma. J Maxillofac Oral Surg 2020; 19: 131-135.

[27] King C, Elsherif N, Kirwan R, et al. Serial step sections at narrow intervals with immunohistochemistry are required for accurate histological assessment of sentinel lymph node biopsy in oral squamous cell carcinoma. Head Neck.

[28] Chone CT, Aniteli MB, Magalhães RS, et al. Impact of immunohistochemistry in sentinel lymph node biopsy in head and neck cancer. Eur Arch Oto-Rhino-Laryngology 2013; 270: 313-317.

[29] Mallo Magariños M, Suárez Ajuria M, Marichalar Mendía X, et al. Diagnostic yield of sentinel lymph node biopsy in oral squamous cell carcinoma T1/T2-N0: systematic review and meta-analysis. *Int J Oral Maxillofac Surg*. Epub ahead of print 2021. DOI: 10.1016/j.ijom.2021.01.020.

[30] Schilling C, Stoeckli SJ, Haerle SK, et al. Sentinel European Node Trial (SENT): 3-year results of sentinel node biopsy in oral cancer. Eur J Cancer 2015; 51: 2777-2784.

[31] Miura K, Hirakawa H, Uemura H, et al. Sentinel node biopsy for oral cancer: A prospective multicenter Phase II trial. Auris Nasus Larynx 2017; 44: 319-326.

[32] Abdul-Razak M, Chung H, Wong E, et al. Sentinel lymph node biopsy for

early oral cancers: Westmead Hospital experience. ANZ J Surg 2017; 87: 65-69.

[33] Crocetta FM, Botti C, Pernice C, et al. Sentinel node biopsy versus elective neck dissection in early-stage oral cancer: a systematic review. Eur Arch Oto-Rhino-Laryngology 2020; 277: 3247-3260.

[34] Hasegawa Y, Tsukahara K, Yoshimoto S, et al. Neck Dissections Based on Sentinel Lymph Node Navigation Versus Elective Neck Dissections in Early Oral Cancers: A Randomized, Multicenter, and Noninferiority Trial. J Clin Oncol 2021; 39: 2025-2036.

[35] Schiefke F, Akdemir M, Weber A, et al. Function, postoperative morbidity, and quality of life after cervical sentinel node biopsy and after selective neck dissection. Head Neck 2009; 31: 503-512.

[36] Den Toom IJ, Boeve K, Lobeek D, et al. Elective neck dissection or sentinel lymph node biopsy in early stage oral cavity cancer patients: The dutch experience. Cancers (Basel) 2020; 12: 1-13.

[37] Yokoyama J, Hasegawa Y, Sugasawa M, et al. Long term-follow-up multicenter feasibility study of icg fluorescence-navigated sentinel node biopsy in oral cancer. Mol Clin Oncol 2020; 13: 1-8.

[38] Van Den Berg NS, Brouwer OR, Klop WMC, et al. Concomitant radio- and fluorescence-guided sentinel lymph node biopsy in squamous cell carcinoma of the oral cavity using ICG-99mTc-nanocolloid. Eur J Nucl Med Mol Imaging 2012; 39: 1128-1136.

[39] Peng H, Wang SJ, Niu X, et al. Sentinel node biopsy using indocyanine green in oral/oropharyngeal cancer. World J Surg Oncol 2015; 13: 1-7.

[40] Honda K, Ishiyama K, Suzuki S, et al. Sentinel lymph node biopsy using preoperative computed tomographic lymphography and intraoperative indocyanine green fluorescence imaging in patients with localized tongue cancer. JAMA Otolaryngol - Head Neck Surg 2019; 145: 735-740.

Chapter 6

Way to Cure Oral Squamous Cell Carcinoma with Theranostics and Nanoparticular Approaches

Sankha Bhattacharya

Abstract

One of the most prevalent forms of oral cancer is oral squamous cell carcinoma (OSCC), a major cause of morbidity and mortality worldwide. Following a definite oral cancer diagnosis, OSCC is typically treated with a multidisciplinary approach including surgery, chemotherapy, and radiation. In contrast, conventional chemotherapy medicines may be ineffective and have a range of side effects. Many techniques have been proved and authorized for treatment and diagnostics of different types of oral cancer, while others are currently being investigated in clinical trials. This book chapter is aimed to explain the current preclinical status of nano-based techniques to successfully diagnose and treat OSCC. This book chapter would also emphasize recent theranostics approaches utilized to cure OSCC. Nanotechnology also improved cancer biomarker detection, making them faster and more sensitive. To overcome these constraints and improve *in situ* drug delivery, various nanoparticles have been employed as innovation drivers.

Keywords: squamous cell carcinoma (OSCC), matrix metalloproteases (MMPs), magnetic nanoparticles (MNPs), HSC-3 cells, vital protein endothelial growth factor (VEGF), C-reactive protein (CRP)

1. Introduction

According to the World Health Organization (WHO), cancer would take the lives of 10.3 million people globally by 2020, and patients with oral cancer would have a five-year survival rate of just 50% globally. Oral cancer is a challenging disease that affects about 600,000 individuals worldwide each year and is linked to a high rate of morbidity and mortality [1–4]. Mouth cancer is a group of tumors that may affect any area of the mouth, including the pharynx and salivary glands, as well as the surrounding tissues [5]. This term, on the other hand, is often interchanged with oral squamous cell carcinoma (OSCC), the most common malignant epithelial tumor of the oral cavity. OSCC is thought to be responsible for more than 90% of all oral neoplasms [6]. According to the current study results, year survival rates for OSCC range from 50 to 60%, depending on a number of factors such as the patient's lifestyle, the timing of diagnosis, and the location of the primary tumor [7, 8]. As a consequence, OSCC is linked with a poor prognosis in the medical community. Patients with OSCC who get conventional therapy have a significant recurrence rate of the illness, regardless of when treatment started (18–76%). The use of biopsy or histology testing to diagnose OSCC is time intensive, resulting in a

delay in treatment initiation, and as a result, a shorter overall survival time period is obtained [9]. Early-stage cancer detection is critical in determining the most suitable treatment for the patient, which has an impact on their total survival, as stated in the previous paragraph. According to recent research studies, the development of cancer is related to the molecular level of particular indicators in tumor tissue and bodily fluids. Molecular biomarkers, which are categorized as genomic, proteomic, and metabolomic profiles of body specimens, are used to identify the existence or absence of a certain malignancy, as well as its spread and recurrence, among other things. Science and technology focused on extremely small objects, and nanoscience and nanotechnology are providing innovative methods to cancer treatment and detection. Site-specific chemoprevention/therapy using nanoparticles for local drug delivery has recently gained popularity. It is a novel method for treating cancer that aims to overcome and minimize the limits of current cancer treatment and diagnostics. It can detect a single cancer cell *in vivo* and administer drugs straight to it. Nanotechnology has also improved the detection of cancer biomarkers [10–12]. Oral cancer is often treated with a multidisciplinary approach that involves surgery, chemotherapy, and radiation. However, conventional chemotherapy medicines may be ineffective and have side effects. Many nanoparticles have been employed as technological drivers of innovation to overcome these constraints. The present book chapter seeks to highlight developments in the use of new methods in the detection and treatment of oral cancer due to the significance of OSCC as a widespread public health issue. The purpose of this book chapter is to describe the current preclinical state of nano-based methods for oral cancer detection and therapy. This review also discussed (a) oral squamous cell carcinoma, (b) OSCC diagnosis using serum and saliva biomarkers, (c) nano-based OSCC biomarker detection, (d) OSCC therapy methods, and (e) nanotechnology techniques for OSCC treatment or diagnostic.

2. Squamous cell carcinoma of the oral cavity

Oral cancers are a group of cancers that develop in different parts of the mouth, each with its own set of risk factors, incidence, and treatment options [13]. Understanding the mechanisms of cancer initiation and progression may aid us in selecting the most appropriate therapeutic strategy at the appropriate time. Despite advances in therapeutic methods, the morbidity and mortality ratios for squamous cell carcinoma of the oral cavity have remained unchanged for the past 30 years. After a carcinogenic insult to the oral cavity, it occurs in several steps, resulting in various molecular changes that disrupt cellular growth, proliferation, and differentiation. The latter is marked by cellular transformation and carcinogenesis. Oral carcinogenesis follows the same pattern as other cancers, starting with a precursor lesion and progressing to localized and metastatic disease [14]. The histological grade of dysplasia indicates how far the disease has progressed from normal to hyperplasia. The most common cellular events discovered in these examinations are changes in nuclear size and shape, enlarged cells and nuclei, enhanced mitotic picture, and increased nucleus/cytoplasm ratio. Elevated cellular density, dyskeratosis, hyperplasia of basal cells, bulbous drop-form of rete pegs, and secondary nodules on rete tips are pathological features of tissues in the later stages of squamous cell carcinoma.

3. Biomarkers in oral squamous cell carcinoma (OSCC)

Early detection of OSCC improves life quality while lowering the cost and side effects of medical treatments. Because OSCC has such a high recurrence

rate, early detection is crucial in determining the disease's prognosis [15]. OSCC remains a significant challenge due to the disease's nature, despite recent advances in this area. As a result, as discussed in this book chapter, monitoring the level of biologic markers with high specificity and sensitivity is a promising diagnostic tool for both primary and recurrent oral cancer detections. According to the National Institutes of Health, biomarkers are indices of normal or pathological conditions that can be reliably and precisely measured [16]. Mutated DNA, mRNA, metabolomes, secreted proteins, and small molecules are examples of biomarkers [17, 18]. A cancer biomarker is a molecule that is secreted by a tumor or produced in response to the onset or progression of cancer. In addition, the ideal biomarker should be a noninvasive method with high-positive and high-negative predictive values that reflect the stage of cancer. As a result, the marker can be used to predict treatment efficacy, diagnosis, and prognosis in cancer patients.

3.1 OSCC salivary biomarkers

Saliva collection is easy and painless, making it a promising biomarker discovery tool. The changes in the saliva genome and protein profile after the onset of cancer or as the disease progresses in oral cancer have been studied in several studies [19, 20]. When collecting, processing, and storing saliva, however, some acquaintances should be considered. Cross-validation is required before extrapolating biomarkers into clinical applications. Different data collection, processing, and analysis techniques could explain the wide range of results seen in studies looking for salivary oral cancer biomarkers. As a result, more research is needed to standardize the aforementioned techniques as well as reference levels in order to obtain valid biomarkers.

3.2 Salivary RNA-base biomarkers

In the mouth, salivary RNAases were supposed to break down RNA. These alluring biomarkers, on the other hand, are obstinate, being carried in apoptotic bodies or actively liberated from cellular vesicles such as exosomes. Oral cancer patients have been found to have low levels of miRNA 200a and miRNA 125a, but significantly higher levels of miRNA 31. Upregulated miRNA 184 and downregulated miRNA 145 have also been linked to malignant oral cancer.

3.3 Salivary protein-based biomarkers

Cancer biomarkers could include oxidative stress markers. Carbonylation causes irreversible protein damage, which leads to cell toxicity. In OSCC patients, the infiltration of reactive radicals into the oral epithelial cells results in a significant increase in salivary carbonyls. Matrix metalloproteases (MMPs) are enzymes that degrade a wide range of proteins. During OSCC, which is highly invasive and metastatic, different types of MMPs have been shown to be significantly altered. MMP-2 and MMP-9 expression in oral cancer patients has been linked to a poor prognosis in the wild. The immune system produces proteins called interleukins (IL). In cellular signaling cascades, naturally occurring proteins play a variety of roles, some of which are critical in cancer. Interleukin-6 (IL-6) and interleukin-8 (IL-8) are two different types of interleukin-6. The prevalence of OSCC has been reported to be on the rise. The cytokeratin fragment 21–1 is a squamous tumor marker (Cyfra 21–1).

4. Serum biomarkers

During tumor development, the release of synthesized markers into the circulation results in an increase in markers in cancer patients' serum, which can be used to predict the development of the cancer itself. The secreted origins of tumor-specific or tumor-associated serum biomarkers are known. Taking regular measurements of these biomarkers could provide valuable insight into how patients respond to anticancer treatments in the long run. Both the progression of the disease and its treatment are discussed in detail. Adiponectin is a protein hormone that can be found in the bloodstream and performs a number of different functions. It aids in the digestion of glucose and fatty acids in the bloodstream. Guo et al. discovered that the levels of adiponectin were higher. The incidence of tongue squamous cell carcinoma (TSCC) has dropped significantly. Hypoadiponectinemia has also been associated with lymphoma in some studies. The prognosis for TSCC metastasis is abysmal. Another protein, hemoglobin (Hb), being associated with an increased risk of oral cancer in the opposite direction. Reduced hemoglobin levels and anemia have been linked to increased tumor oxygenation and lymph node metastasis, and Hb corrections have been shown to improve prognosis in cancer patients.

Low hemoglobin levels indicate advanced OSCC, especially in larger tumors requiring more radial treatment. Pro-inflammatory cytokines are a group of small proteins that play a role in inflammation. Oral cancer is the most common link. In a prospective study, Schiegnitz et al. looked at the role of IL-6, 8, soluble IL-2 receptor (SIL-2R), MHC class I polypeptide-related sequence B (MICB), and tumor necrosis factor alpha (TNF-a) in OSCC. According to their findings, all of these biomarkers showed an upward trend in serum samples from oral cancer patients. Promising OSCC prognostic indicators have been identified as IL-6 and SIL-2R. Chang et al. also discovered a link between oral cancer clinical manifestations and blood levels of 12 cytokines. However, the results of a different study refuted these assertions. Individual or panels of serum cytokines are not appropriate oral cancer biomarker candidates due to these discrepancies and heterogeneous literature searches. However, it has been suggested that some inflammatory and immune system-related proteins are self-contained. Oral cancer metastasis indicator C-reactive protein (CRP), for example, is an IgG analog that stimulates the release of pro-inflammatory cytokines. CRP is an inflammatory marker that has been linked to a variety of diseases and cancers. Previous research has looked into the relationship between OSCC size, stage, and subsequent survival and CRP serum levels. Elevated serum CRP has been proposed as a predictor of poor prognosis and a low survival rate in oral cancer patients. The soluble protein Decoy receptor 3 (Dcr3) prevents programmed cell death. It is a member of the TNF receptor family. Several cancers have been found to have a high rate of cell death and amplification. According to estimates, when lymph node metastasis occurs at the same time as OSCC, the prognosis is worsened. In this context, serum MMPs have also been investigated. Liu et al. investigated the link between MMP-9 and pathological manifestations in oral cancer patients in a survey. A higher level of this factor was discovered to be linked to a shorter life expectancy. On the other hand, some of these serum proteins have been suggested as potential biomarkers for OSCC diagnosis but not prognosis prediction. For example, MMP-3 levels have not been linked to OSCC clinicopathological features. As potential biomarkers, different levels of growth factors in the serum of oral cancer patients have been proposed. Vascular. In various tumor propagation processes, the vital protein endothelial growth factor (VEGF) is thought to induce angiogenesis. Nodal metastasis and advanced OSCC have been linked to higher levels of this factor. Furthermore, in a 6-month cumulative survival study, the results of a 12-month study OSCC serum insulin-like growth factor (IGF) and survival were found to be strongly linked.

5. OSCC treatment strategies based on nanomaterials

Low levels of cancer biomarkers in tumor tissue or body fluids, a narrow margin between non-cancerous and cancerous samples, and the sensitivity of measurement assays are all major roadblocks to precise cancer biomarker detection. As a result, the ideal biomarker is one that can detect even the tiniest tumor cells using specific biomarkers before cancer progresses and clinical symptoms emerge. Previously, a variety of methods for measuring multiple biomarkers were developed, with immunoassays like ELISA becoming the most popular technique for protein detection due to low detection limits. However, this area has some limitations, including a long detection time, high costs, and a small sample size. Some novel approaches are currently being used. Polynucleotide barcodes, multiplexed bead platforms, and microarrays, for example, have superior limits of detection but are costly and require technical expertise. They are widely available in clinics and are efficient and cost-effective methods for detecting cancer biomarkers, despite scientists' attempts to put in place some quick and sensitive measures. Nanotechnology, for example, is gaining popularity among scientists in this field. In a study by Sungyub et al., gold nanoparticles were used to conjugate with DNA probes in oral cancer salivary samples. S100 calcium-binding protein P (S100P) mRNA was found to have a detection limit of 3 nM as a potential OSCC biomarker. The use of nanotechnology in the *in vitro* treatment of oral cancer is demonstrated in **Figure 1.**

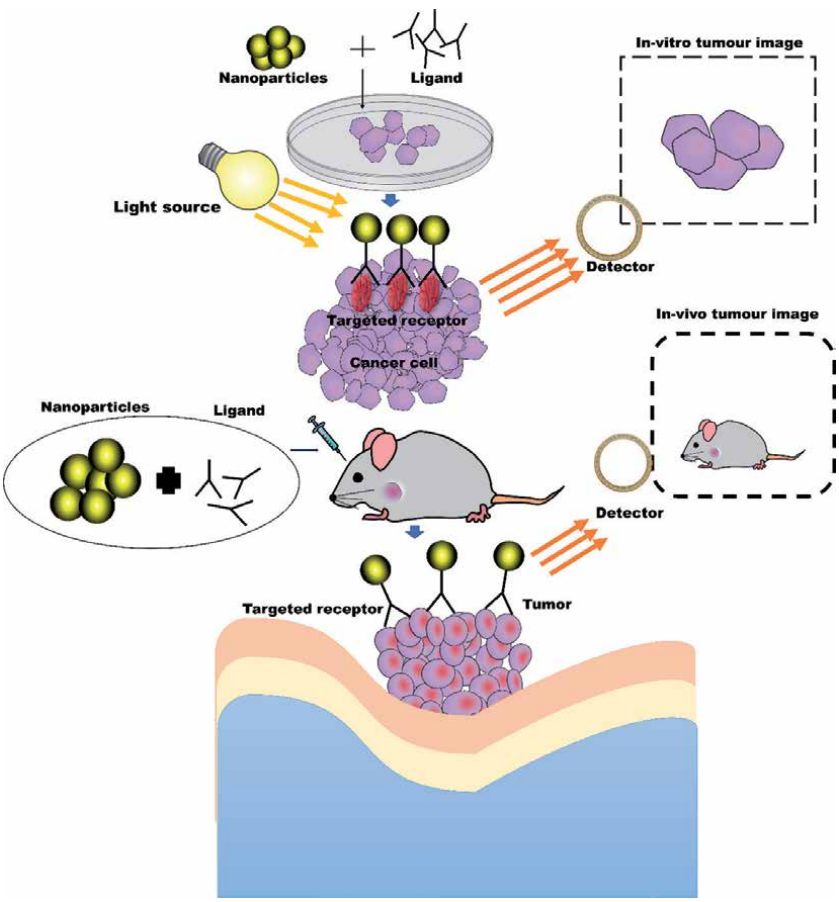

Figure 1.
In vitro *and* in vivo *imaging of oral cancer using nanotechnology has been demonstrated.*

6. OSCC treatment strategies based on nanomaterials

Traditional therapeutic approaches to treating OSCC are associated with a number of side effects that can be both temporary and permanent. Oral carcinogenesis has been shown to respond well to novel treatment approaches. This group could include therapeutic molecules such as siRNAs and various active targeting ligands. Several natural products have also shown promise in the treatment of oral cancer by interfering with various cell signaling pathways such as free radical scavenging, inhibiting the formation of DNA adducts, and regulating the properties of apoptosis-related genes. However, these compounds' low bioavailability and solubility have limited their clinical application. As a result, it is critical to improve preventative and therapeutic strategies. For the development of drug delivery systems, the time of drug contact with oral tumor cells is an important consideration. As a result, a sustained and targeted mucoadhesive drug delivery system into the oral cavity has been developed. The residence time of nanoparticles can improve drug mucus interaction and result in better results. The studies also revealed that local delivery of nanoparticles can be used for site-specific chemoprevention and therapy. Nonspecific drug uptake is reduced by cells to improve drug targeting into the oral cancer site. The reticuloendothelial system (RES) and improved plasma half-life, which result in lower drug dosage, are two other advantages. The aspects of cancer therapy are based on nanoparticles. According to scientific reports, various types of nanoparticles have been tested for cancer therapy. Some of the most recent nanoparticles that have been tested for the treatment of drug-resistant cancer cells include magnetic nanoparticles (MNPs), liposomes, polymeric nanoparticles, gold (Au) nanoparticles, and nano-diamonds. Various nanotechnology approaches can be used to selectively target cancer biomarkers and cancer cells. The use of specific crosslinkers against cancer cells, such as antibodies or aptamers, can also facilitate the development of early detection methods. **Figure 2** summarizes the potential therapeutic approaches.

Oral oncogenesis is a type of cancer that starts in the mouth and spreads to the rest of the body. Nanocarriers are also being developed that are functionalized with various targeting agents (ligands, tumor-associated proteins). Antigens, antibodies, and aptamers have shown promise in improving the cancer cell delivery of a specific target. In a variety of main cancer immunotherapy or target drug/gene delivery pathways, this strategy can use a single agent or a combination of agents. It can be viewed as a primary strategy involving specific interactions between the nanocarrier and receptors on the target cancer cell, which could promote nanocarrier internalization endocytosis through receptors. A basic understanding of cell biology, tumor biology, and immunology is required for the rational design of NPs for cancer therapeutics, and advances in nanotechnology will be heavily reliant on advances in cancer biology. Magnetic nanoparticles (MNPs) are one of the most researched nano-delivery systems in cancer treatment. The MNPs showed high efficiency and ideal drug loading when coated with oleic acid and embedded with anticancer agents such as doxorubicin and paclitaxel, according to the reports. Various researchers have investigated them for therapeutic approaches such as hyperthermic therapy, that is, magneto hyperthermia (MHT). The toxicity of this method was reduced while the specific lysis of tumor cells was increased. Candido et al. investigated the effects of polyphosphate-coated MNPs on human OSCC (UM-SCC14A). Their findings showed that cancer treatment with magneto hyperthermia can be effective related to cell death in the target cancer tissue According to the authors, there were two main findings from MNP-based magneto hyperthermia treatment: a high level of apoptosis and fibrosis, as well as an inhibition effect on cell proliferation. As a carrier for anticancer drug delivery, polymeric nanoparticles

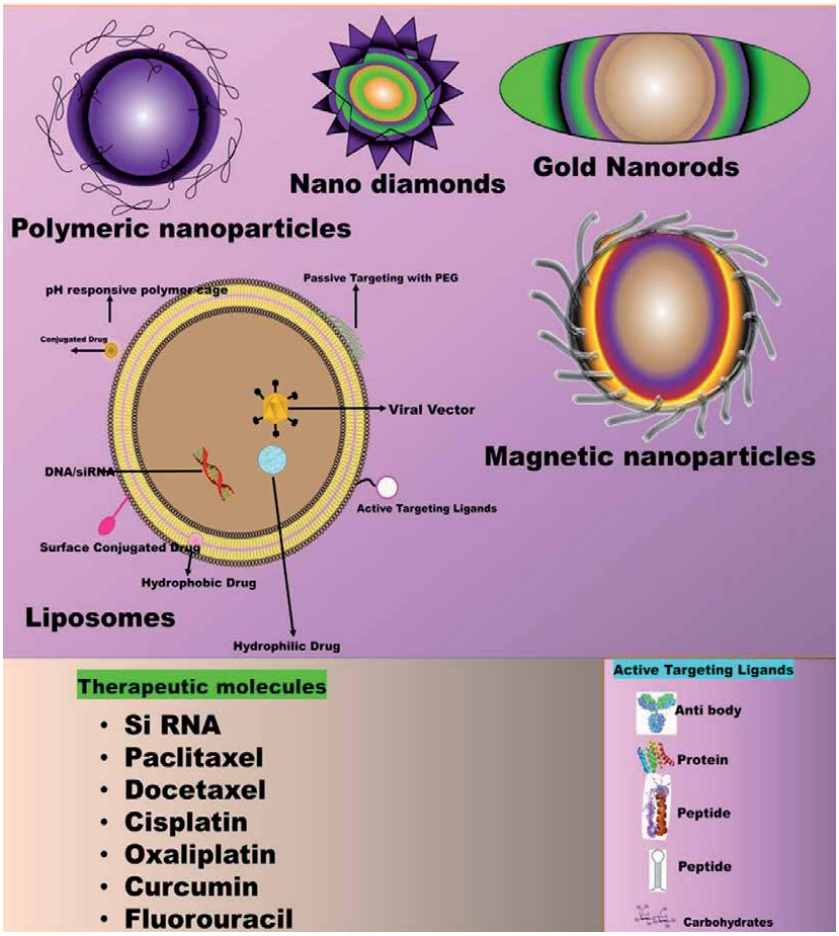

Figure 2.
There have been advancements in the treatment of oral cancer. A wide range of drugs and/or therapeutic molecules are used to treat oral cancer. Nanocarriers show promise in the development of effective oral oncology therapy, in addition to active targeting ligands.

have been shown to be impactful. It is one of the most widely used nanoparticles *in vitro* and *in vivo*. However, before they can be used in clinical trials, they must overcome a number of challenges. In addition to low molecular weight drugs, polymeric nanoparticles have demonstrated the ability to transport macromolecules such as proteins and genes. Hydrophilic polymers, such as polyethylene glycol (PEG), aid in the stabilization of nanoparticles, which improves drug targeting into cancer sites by reducing nonspecific drug uptake by cells, according to the studies. These nanoparticles are less toxic, have a higher level of stability, and have a higher loading capacity, according to reports. Because of the properties of drugs that are not soluble in water, the use of biodegradable polymers has increased dramatically in recent years, such as increased plasma half-life and reticuloendothelial system inhibition of fast clearance (RES). Biodegradable linkages can be used as a backbone to assist in the formation of high-surface-area nanoparticles. In the case of apoptosis induction in SCC-9 human OSCCs, PCL nanoparticles with curcumin were coated with chitosan, which showed promising outcomes of this research. When Sulfikkarali et al. used the nanoprecipitation method to create naringenin-loaded polymeric (Eudragit E) nanoparticles and tested their anticancer activity in hamster carcinogenesis, they discovered that they had a significant anticancer effect. The prepared nanoparticles (with an average size of 90 nm) had an encapsulation

efficiency of 88%, indicating that they were effective at encapsulating their surroundings. They discovered that a polymeric drug-loaded nanoparticle improved the anticancer efficacy of naringenin and had better antilipid peroxidative, antiproliferative, and antioxidant properties than the free drug. When developing controlled-release mucoadhesive drug delivery systems, the time at which the drug comes into contact with oral tumor cells is an important factor to take into account. The development of a long-acting, targeted mucoadhesive drug delivery system for the oral cavity may be advantageous as a result. To create a mucoadhesive patch of methotrexate (MTX)-loaded liposomes for targeted delivery in OSCC, Jin et al. used the thin film hydration method, which they developed in-house. The liposomes that were prepared had a mean particle size of 105 nm and a loading efficiency of 54%, respectively. Using an MTT assay, HSC-3 cells were utilised to examine the cytotoxicity of the liposomes that had been generated. The mucoadhesive buccal patches had appropriate bioadhesive qualities as well as the potential to offer sustained MTX release, according to the scientists. According to their findings, oral mucoadhesive patches for oral cancer can be utilised as a primary strategy to bypass the constraints of targeted delivery in oral cancer chemotherapy, lowering the required dose while reducing drug toxicity. According to the researchers' findings, the nanoparticles they prepared had a pro-oxidant effect in HSC-3 cells due to the high levels of ROS present in their experiments. Due to the ease with which they can be prepared, their high biocompatibility, and their ideal functionalization properties, gold (Au) nanoparticles are gaining attention in the field of cancer therapy. It is possible that their ability to conjugate with other biomolecules without altering their biological properties will prove to be a very useful option for the treatment of oral cancer, having the ability to conjugate a wide range of mucoadhesive substances. The combination of biopolymers and the outstanding properties of Au nanoparticles, such as their non-cytotoxicity, has resulted in their widespread application, which is used in a variety of biomedical applications and drug delivery systems to treat a variety of oral cancer cells of different types. Furthermore, these nanoparticles are one of the most well-known cancer nanoparticles due to their surface plasmon resonance, which is a property of surface plasmon resonance. The importance of early detection cannot be overstated. In order to improve the early detection of diseases such as cancer, as previously stated, many studies are being conducted and expanded. For bio-imaging and diagnostic applications, nano-diamonds have attracted considerable attention due to their low toxicity, ideal surface properties, and stable fluorescence that does not fade when exposed to ultraviolet light. These nanoparticles can also be used to immobilize proteins, making them excellent candidates for local drug delivery into oral diseases such as oral cancer.

7. Conclusion

Oral cancer is a type of cancer that primarily affects oral epithelial cells, but it can also spread to other parts of the body and be fatal. The most common type of cancer is OSCC, which accounts for more than 90% of all oral cancers. OSCC remains a serious public health concern despite extensive research because of its poor prognosis . Novel chemoprevention technologies are becoming increasingly important as traditional therapeutic methodologies are frequently insufficient. Nanotechnology has proven to be extremely beneficial in this case. Nanoparticle-based diagnostic methods for OSCC detection and diagnosis are capable of providing real-time, appropriate, and cost-effective diagnoses. They can show molecular-targeted imaging, nano-scale biomarker analysis, and post-treatment OSCC prediction. Bioconjugate nanoparticles have a wide range of applications for

amplified transduction of biomolecular recognition events, as demonstrated by the studies described above. Because of their optical and electrochemical applications, such nanoparticle labels serve as the foundation for ultrasensitive protein and nucleic acid assays. This book chapter summarized the most recent advances in nanoparticles for oral cancer diagnosis and treatment. Nanoparticles have been studied for their unique physicochemical properties, such as their ultrasmall size, high reactivity, and ability to be functionalized. It has been demonstrated that accurate and timely oral cancer diagnosis tools, as well as highly effective oral cancer treatment strategies, exist. Nanoparticles can be used to visualize oral cancer, deliver therapeutic agents to tumors selectively, and destroy tumors by using a variety of therapeutic techniques. Hybrid systems, in particular, will garner more attention because they provide nanoparticles with a flexible platform for achieving bio-multifunctionality. The use of nanomedicine in the modern diagnosis and treatment of oral cancer is very exciting. Despite the fact that nanoparticles have a wide range of potential applications in the treatment and prevention of diseases, the nanomedicine field is currently limited in its use of nanoparticle technologies in the prevention and treatment of oral cancer. The studies in this collection focused on therapeutic activities, which were mostly conducted *in vitro* or in preclinical oral cancer models. Because of the complex pathophysiology of oral cancer, such as abnormal hemodynamics, the pharmacokinetics and biodistribution of therapeutic agents can vary, leading to misleading results. Because of the high sensitivity of the new nanoparticle-based sensing protocols, they can detect disease markers, biothreat agents, and infectious agents that are not detectable by traditional methods. Methods of the past early disease detection or terrorist attack warnings could be provided by such highly sensitive biodetection schemes. Although the use of nanoparticle tags for protein detection is still in its early stages, the lessons learned in ultrasensitive DNA detection should be useful. Nonspecific adsorption issues, which frequently limit bioaffinity assay detectability, must be carefully considered for the successful implementation of the new signal-amplify cation strategies. Proper washing and surface blocking steps should be used to avoid amplifying the cation of background signals (associated with non-specific adsorption of the nanoparticle). Although there were *ex vivo* studies of tissue and saliva samples, as well as *in vivo* training in animal models, more experiments must be applied before these strategies can be implemented. In the near future, nanobiosensors are expected to play a larger role in electroanalytical science. For clinical applications, protocols for the synthesis and functionalization of nanoparticles must be developed. Early disease detection, genetic mutations, and bio-targets appear to have the greatest implications. Nanomaterial-based biosensors offer quick, simple, and sensitive cancer detection systems that could be useful in anticancer biosensor research.

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Competing interests

The author declares that the author has no competing interests.

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References

[1] Wei R, Qiu H, Xu J, et al. Expression and prognostic potential of GPX1 in human cancers based on data mining. Ann Transl Med. 2020;8(4):124. doi:10.21037/atm.2020.02.36

[2] Liu Q, Li S, Dupuy A, et al. Exosomes as New Biomarkers and Drug Delivery Tools for the Prevention and Treatment of Various Diseases: Current Perspectives. Int J Mol Sci. 2021;22(15): 7763. Published 2021 Jul 21. doi:10.3390/ijms22157763.

[3] Van DN, Van LP, Tien HN, Van ND, Le Van Q. Multidisciplinary Therapy Management for Locally Advanced Minor Salivary Gland Cancer in Oral Cavity: A Case Report and Literature Review [published online ahead of print, 2021 Jul 19]. Ear Nose Throat J. 2021;1455613211031026. doi:10.1177/01455613211031026.

[4] Al-Jamaei AAH, van Dijk BAC, Helder MN, Forouzanfar T, Leemans CR, de Visscher JGAM. A population-based study of the epidemiology of oral squamous cell carcinoma in the Netherlands 1989-2018, with emphasis on young adults [published online ahead of print, 2021 Mar 24]. Int J Oral Maxillofac Surg. 2021;S0901-5027(21)00101-6. doi:10.1016/j.ijom.2021.03.006

[5] RaziyehsadatRezvaninejad H. Evaluation of Relationship between ABO bloods with Oral Cancer and Compared with Healthy Individuals. Annals of the Romanian Society for Cell Biology. 2021 Jul 16;25(6):17712-20

[6] Vassiliou LV, Acero J, Gulati A, et al. Management of the clinically N0 neck in early-stage oral squamous cell carcinoma (OSCC). An EACMFS position paper. J Craniomaxillofac Surg. 2020;48(8):711-718. doi:10.1016/j.jcms.2020.06.004.

[7] Wushou A, Wang M, Yibulayin F, et al. Patients With cT1N0M0 Oral Squamous Cell Carcinoma Benefit From Elective Neck Dissection: A SEER-Based Study. J Natl Compr Canc Netw. 2021;19(4):385-392. Published 2021 Jan 29. doi:10.6004/jnccn.2020.7632.

[8] Jardim JF, Galvis MM, Fabelo IR, Soares FA, Pinto CA, Kowalski LP. Intratumoral lymphatic vascular density is an independent factor for disease-free and overall survival in advanced stage oral squamous cell carcinoma. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2021 Jul 21.

[9] Narayanan MS, Kassim NK, Liszen T, Abdullah B, Omar J, Hairon SM, Lazim NM. The Utility of Beta 2 Microglobulin (B2M) as an Initial Diagnostic Tool for Oral Squamous Cell Carcinoma (OSCC): Evidence from a Malaysian Scenario. Bangladesh Journal of Medical Science. 2019 Aug 30;18(4):729-35.

[10] Singhal J, Verma S, Kumar S, Mehrotra D. Recent Advances in Nano-Bio-Sensing Fabrication Technology for the Detection of Oral Cancer. Mol Biotechnol. 2021;63(5):339-362. doi:10.1007/s12033-021-00306-x.

[11] Fang Z, Zhao J, Xie W, Sun Q, Wang H, Qiao B. LncRNA UCA1 promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by sunppressing miR-184 expression. Cancer Med. 2017;6(12):2897-2908. doi:10.1002/cam4.1253

[12] Durgalakshmi D, Rishvanth R, Mohanraj J, Aruna P, Ganesan S. A Roadmap of Cancer: From the Historical Evidence to Recent Salivary Metabolites-based Nanobiosensor Diagnostic Devices. Current Metabolomics and Systems Biology Formerly: Current Metabolomics. 2021 Mar 1;8(1):27-52.

[13] Barrett TF, Mazul AL, Stepan KO, Wood CB, Paniello RC, Zevallos JP, Massa S, Jackson RS, Schmitt NC, Zenga J, Kang SY. The role of age in treatment decisions for oral cavity squamous cell carcinoma: Analysis of the National Cancer Database. Oral Oncology. 2021 Jul 1;118:105330.

[14] Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6(1):92. Published 2020 Nov 26. doi:10.1038/s41572-020-00224-3

[15] Capote-Moreno A, Brabyn P, Muñoz-Guerra MF, et al. Oral squamous cell carcinoma: epidemiological study and risk factor assessment based on a 39-year series. Int J Oral Maxillofac Surg. 2020;49(12):1525-1534. doi:10.1016/j.ijom.2020.03.009

[16] Durham JS, Brasher P, Anderson DW, et al. Effect of Fluorescence Visualization-Guided Surgery on Local Recurrence of Oral Squamous Cell Carcinoma: A Randomized Clinical Trial. JAMA Otolaryngol Head Neck Surg. 2020;146(12):1149-1155. doi:10.1001/jamaoto.2020.3147

[17] Loktionov A. Biomarkers for detecting colorectal cancer non-invasively: DNA, RNA or proteins?. World J Gastrointest Oncol. 2020;12(2):124-148. doi:10.4251/wjgo.v12.i2.124.

[18] Huda MN, Nafiujjaman M, Deaguero IG, et al. Potential Use of Exosomes as Diagnostic Biomarkers and in Targeted Drug Delivery: Progress in Clinical and Preclinical Applications. ACS Biomater Sci Eng. 2021;7(6):2106-2149. doi:10.1021/acsbiomaterials.1c00217.

[19] Buzalaf MAR, Ortiz AC, Carvalho TS, et al. Saliva as a diagnostic tool for dental caries, periodontal disease and cancer: is there a need for more biomarkers?. Expert Rev Mol Diagn. 2020;20(5):543-555. doi:10.1080/14737159.2020.1743686.

[20] Campanati A, Martina E, Diotallevi F, et al. Saliva Proteomics as Fluid Signature of Inflammatory and Immune-Mediated Skin Diseases. Int J Mol Sci. 2021;22(13):7018.

Chapter 7

Radiotherapy in Oral Cancers: Current Perspective and Future Directions

A.S. Kirthi Koushik and Ram Charith Alva

Abstract

Oral cancers form one of the most common malignancies seen worldwide, with a steady increase in number over time. Surgery with the addition of adjuvant therapy forms the cornerstone of therapeutic management for these cancers. Despite excellent surgical management, loco-regional recurrences have always been of concern. This has expanded the role of radiotherapy, with concomitant therapies, allowing to establish an effective management protocol. Over the last two decades, there have been huge strides taken towards understanding these specific aspects and providing insight into the most fruitful application of radiotherapy in these patients. In this chapter, we have presented the oncologists perspective to dealing with the non-surgical aspects of oral cancer management. We have elaborated on the chronological order with which radiotherapy has evolved and provided the contemporary aspects of decision making, essential for current practice. The evidence-based approach will address all components of radiotherapy workflow from basic understanding of patient's anatomy, planning & evaluation during therapy to the outcomes & toxicity profiles to be expected in day-to-day clinics. Established guidelines have been incorporated into the graphical representations to ensure scenario-based understanding. Future perspectives, essential for identifying the possible direction of therapy & potential improvements in outcomes, have also been addressed.

Keywords: Oral cancers, Radiotherapy, Adjuvant, Definitive, Brachytherapy, Chemotherapy

1. Introduction

Oral cancers are one of the most well described & clearly detailed cancers in history, since the times of ancient Egyptian and Ayurveda (Sushrutha) systems. Globally, as per the data released from International Agency for research on cancer (IARC), oral cancer occurs most commonly in middle- & low-income countries with worldwide incidence of 377,713 cases with 177,757 deaths seen in 2020 [1]. The worldwide incidence rate is 4.1 cases per 100,000.

Since the evolution of modern contemporary management for oral cavity cancers, radiotherapy has played a pivotal role along with surgery and recently with chemotherapy in improving patient outcomes. After the milestone discovery of x-rays by Wilhem Roentgen in 1898, utility of radiation in various cancers was established. The role of radiotherapy in oral cancers specifically, developed between the two world wars, as the morbidity was less troublesome and was easier to deliver

in community hospitals [2]. However, as surgical processes got more flexible and easier to execute and the survival rates of patients receiving radiation alone were not very encouraging, radical surgery resurfaced as the primary modality for oral cancers mid-century. Also, concerns of long-term radiation morbidity affecting patient's quality of life, in those receiving conventional radiation as a sole modality came to the fore. Keeping this in mind, towards the end of the 20th century, development of newer technology was directed towards minimising these particular issues. Also, optimum and effective combinations between surgery & radiotherapy with or without the addition of chemotherapy, were slowly established as standards of care which have been thoroughly reviewed in this chapter.

In order to keep the options simple and to make the life of an oncologist simpler, one can divide oral cancers as early, locally advanced and metastatic disease. In this chapter, we will give an overview of the current understanding for the choice of treatment in these different categories with insight into the specific therapeutic aspects of both external & internal radiation therapy. We will also dwell upon the advances in recent research related to oral cancer management as well as the possible future directions from the prism of the new age radiation oncologist.

2. Basic aspects in oral cavity cancers

2.1 Anatomy from an oncologist perspective

In order to understand a disease, one needs to be well versed with the anatomy of the area and since we want to know the way this would influence a radiation oncologist's outlook, the following section will address anatomy from a radiation oncologists' perspective (**Figure 1**).

Oral cavity includes lips, buccal mucosa, gingivobuccal sulcus, superior and inferior alveolar ridges, teeth & mandible, oral tongue, floor of mouth (FOM),

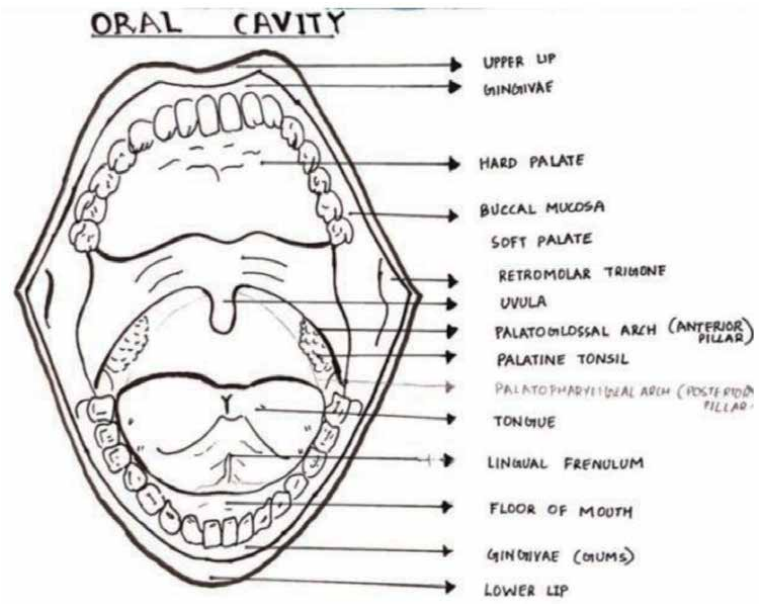


Figure 1.
Anatomical aspects of oral cavity.

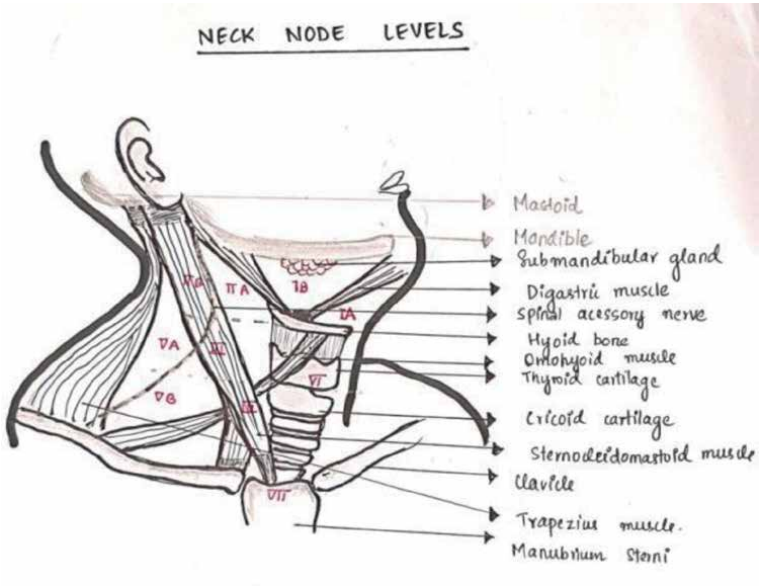


Figure 2.
Description of various nodal levels in the neck.

retromolar trigone (RMT) and hard palate [3]. One needs to know a few terminologies like buccal mucosa which means mucosal surface of lips and cheek, whereas gingival mucosa means mucosal lining of teeth, alveolar arches and gums. The area of maximum incidence of squamous cell carcinoma is at the gingivobuccal sulcus which is the junction of buccal mucosa to gingival mucosa. Floor of mouth is the U-shaped sling formed due to joining of two mylohyoid muscles to a fibrous median raphe. Oral tongue also known as Anterior 2/3rd of tongue includes the tip, lateral border and body which consists of intrinsic and extrinsic muscles. It is imperative to know the muscles as their involvement can upstage the disease, which will be discussed later in the chapter. Intrinsic muscles have no bony attachment and are divided into longitudinal, transverse and vertical groups. Extrinsic muscles do have a bony attachment and originate outside the tongue, and are made up of four paired bundles - genioglossus, hyoglossus, styloglossus and palatoglossus. These can be appreciated well on a CT scan and an MRI scans.

The most important feature of neoplasia is the potential for spread and the most common route of spread in oral cavity tumours is the lymphatic spread (**Figure 2**). Since radiotherapy is a branch which tackles locoregional disease it becomes imperative to have a thorough knowledge of the lymphatic drainage. In order to understand this better, we are dividing the lymphatic channels as functional pathways:

1. Main lymphatic pathway: submental ➜ submandibular ➜ anterior jugulodigastric ➜ middle and lower deep cervical nodal groups ➜ jugular collecting trunk

2. Posterior accessory pathway: posterior part of jugulodigastric group ➜ middle and deep posterior cervical groups

3. Anterior lymphatic pathway: submental and jugulo-omohyoid ➜ lower internal jugular nodes.

4. Superficial lateral pathway: occipital and mastoid group.

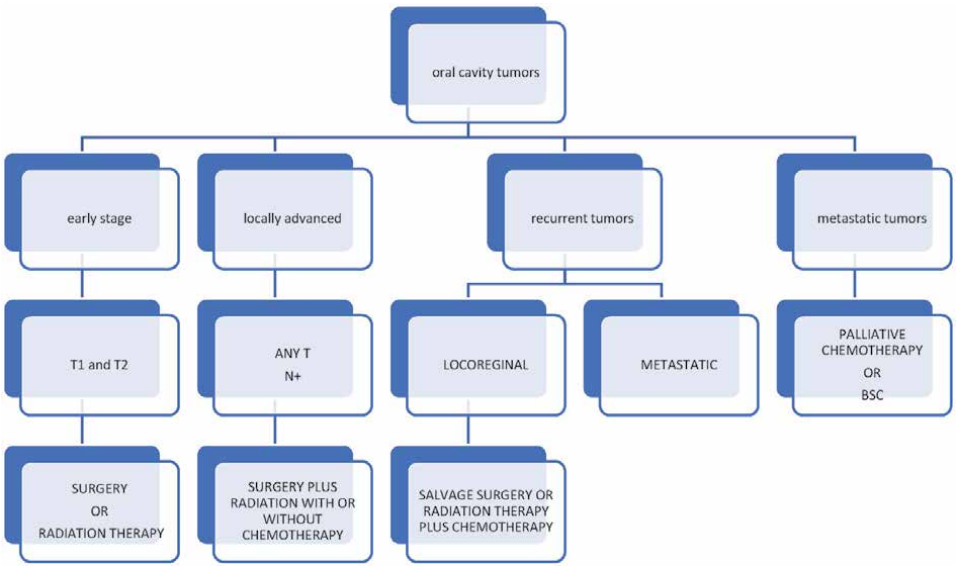


Figure 3.
Overview of management of Oral cancers.

2.2 Basic principles of treatment and broad guidelines

Radiotherapy is loco-regional form of treatment just like surgery. In oral cavity cancers one needs to keep in mind the functionality and cosmesis when deciding on the management, hence it requires a multidisciplinary team to take a call. For management and prognosis reasons, these tumours are divided in to early stage, locally advanced and metastatic tumours. The flow chart provides concise & definitive steps on how to manage oral cavity tumours (**Figure 3**).

For any malignancy there are only three weapons and they are surgery, radiation therapy and systemic therapy. The sequencing and need of each therapy are a point of debate. However, in oral cavity tumours, mostly all modalities are required and sequencing is usually pre-determined and rationale is also established.

2.2.1 Early stage

As per TNM this group includes cT1 and cT2, which means localised tumours only. This group is usually tackled by surgery or radiation therapy and role of systemic therapy is limited (**Table 1**).

	Surgery	Radiation therapy	Rationale
Lip	Yes	Yes	Cosmetically better
Buccal mucosa	Yes	Yes	If localised and margins are clear
Tongue	Yes	No	Usually aggressive and depth can be better made out on hpe than mri
FOM	No	Yes	As organ preservation is better
RMT	No	Yes	As posterior margin will not be an issue

Table 1.
Rationale for application of radiotherapy in early oral cancers.

	Surgery +/− Radiation therapy	**Rationale**
LIP	Surgery followed by Radiation +/− Chemotherapy	As locally advanced one modality will not be able to achieve cure.
BUCCAL MUCOSA	Surgery followed by Radiation +/− Chemotherapy	Locally advanced diseases tend to recur locally
TONGUE	Surgery followed by Radiation +/− Chemotherapy	Usually aggressive and tend to recur locally as well as systemically
FOM	Radiation and chemotherapy	As organ preservation is better
RMT	Radiation and chemotherapy	As posterior margin will not be an issue

Table 2.
Rationale for combined modality therapy in advanced oral cancers.

2.2.2 Locally advanced disease

As per TNM this group will include cT1-4 and N+ disease. Basically, it means that the disease has spread to nodes and or direct extension to surrounding structures. This group has to be addressed by combined modality therapy and there will always be some amount of deliberation to be had about the sequencing of the three modalities (**Table 2**).

Recurrent tumours: a tumour can be suggested to have recurred if there is disease recurrence after a period of documented complete remission and usually it is taken as 2 follow-ups of 6 weeks apart with a period of 6 months post primary therapy. Based on this they can be:

1. Locally recurrent: the plan of treatment will depend on the primary therapy received and also the operability. Usually, it is important to reassess the disease to take a call on treatment. If the disease is operable, salvage surgery gives best results. If more than 2 years has elapsed post primary therapy, then radiation can also be advised based on site of recurrence.

2. Locoregionally recurrent: if the duration post primary therapy is more than 2 years and if operable then both salvage surgery with radiation can be offered or else based on the status the treatment can be tailored.

3. Metastatic: these are usually managed with palliative systemic therapy and role of radiation is limited to palliation to relieve the symptoms such as bleeding, pain or dysphagia.

In the following section we are going to address the basis of radiation, how it is delivered and what are the relevant toxicities encountered.

2.3 Basis for radiation

2.3.1 What is radiation?

One needs to remember that discovery of many great things happened by accident. Same thing holds good for practice of radiotherapy, as its use was established when Henry Becquerel left 200 mg of Radium in his vest pocket for 6 hours and later noted ulceration along the adjacent skin. This event opened the eyes of the

scientific community towards the clinical effects of radiation & radioactive sources. From then on, there were many experiments done to establish the role of radiation in treatment of malignancy.

Radiation is nothing but energy in the form of waves or stream of particles which can be explained by emission, propagation and absorption of energy. In the present day, most common form of radiation in use is X-rays though there are utilisation of gamma in brachytherapy and proton in particle therapy but in order to restrict ourselves to common terminologies we are not going in detail about them. This form of radiation is considered to have the ability to produce ions in the cells of tissues it passes through, by dislodging the electrons from atoms, and hence it is commonly called as ionising radiation.

If one needed to know the most basic principle about radiation, it would be that - "radiation is effective only on dividing cells". There are 5 phases that each cell goes through from G0 ➔ G1 ➔ S ➔ G2 ➔ M. Among these G2 & M phases are the most radiosensitive and S phase is the most radioresistant phase. The knowledge of these help in deciding on combination therapy and use of radiosensitisers. Radiation is usually delivered in a fractionated manner and it would be paramount to have the knowledge of the 4R's of radiation therapy to understand the basis for this fractionation:

- Repair: differential rate of repair between tumour & normal tissue cells, is what is utilised in fractionation
- Repopulation: this helps in normal tissues such that if one cell dies, then due to compensatory mechanisms other cells divide rapidly to replace this loss.
- Reoxygenation: oxygen is the most important component for both cell survival and also for effective action of radiation.
- Redistribution: as cell death happens there will be redistribution, making the cells in less radiosensitive phases to move to more radiosensitive phases, thus making radiation very effective and forms another basis for fractionation.

2.3.2 How is it delivered?

One can divide this based on technology, based on setting and based on type. The following flowchart will briefly describe about it (**Figure 4**).

Description of these commonly employed terminology are as follows

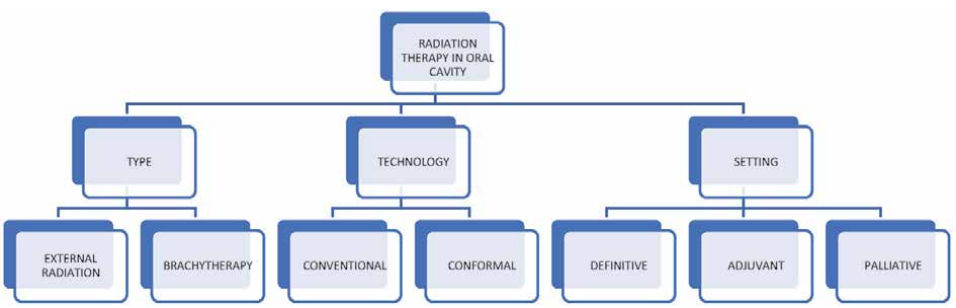


Figure 4.
Aspects of radiotherapy in Oral cancers.

1. External radiation: radiation delivered from a distance away from the body is called external radiation and the unit that's commonly employed for this are known as linear accelerators. There are different types of radiation that are used such as X-rays, gamma rays and particle therapy like protons.

2. Brachytherapy: delivery of radiation in close proximity to or within the target tissue is known as brachytherapy. The usual sources used are Iridium192 and Cobalt60.

3. Conventional: it is the traditional way of radiation delivery, where the dose is delivered without much conformity and is based mainly on bony landmarks.

4. Conformal: it is a contemporary form of radiation delivery where in the conformity is good and avoidance of normal structures can be achieved.

5. Definitive: it means radiation is the main modality of treatment and it is used with an intent to cure.

6. Adjuvant: it means radiation is delivered post-surgery in order to mop up the most probable microscopic disease that is present.

7. Palliative: as the name indicates is to only tackle the symptoms and give relief.

3. Evidence based review on current management concepts in oral cavity cancers

3.1 Risk factors for locoregional recurrence

3.1.1 Risk factors which have been utilised traditionally

Oral cavity cancers are commonly managed with single modality treatment in early stages and a combined modality approach in advanced stages. Radical, oncologically-sound surgical approaches form the backbone of early cancers with a meticulous assessment of the histopathological specimen. Even with appropriate excisions, local and regional recurrences are an extremely concerning aspect which can alter the eventual outcomes in these cancers.

Many retrospective studies and single institution prospective trials demonstrated the benefit of adding radiation adjuvantly for oral cancer patients who have undergone radical surgery. Cooper et al. collated data from RTOG #85-03 & #88-24 to retrospectively sort into 3 risk groups of presumed progressive risk to enable adjuvant treatment decisions [4]. Group I included fewer than 2 involved nodes, no ECE & negative surgical margins. Group II included at least 2 involved nodes or presence of ECE of tumour with uninvolved margins. Group III included microscopically involved surgical margins. Accordingly, comparing outcomes in groups I to III, the loco-regional recurrence rates at 5 years was 17%, 27% & 61% and median survival was 5.6 yrs., 2.6 yrs. and 1.5 yrs. respectively.

Langendijk et al. used the Classification and Regression Tree(CART) or the Recursive Partitioning Analysis(RPA) method to construct homogenous subgroups of well-known prognostication parameters to be able to identify locoregional recurrence risk [5]. Accordingly, 801 patients were divided into RPA 1 group (Intermediate risk) which included no ECE and free surgical margins; RPA 2 (High Risk) which included T1,T2 and T4 tumours with close or positive surgical margins

or one positive node with ECE and RPA 3 (Very High Risk) which included T3 tumours with close or positive margin, N3 neck or multiple nodes with ECE. The 5-year LRC was 92%, 78% and 58% and OS rates were 67%, 50% and 36% for the three RPA classes respectively.

Salama JK et al. used pooled multivariate analysis to give a consensus statement on use of adjuvant therapy in head and neck cancers [6]. They divided patients into two groups to identify their risk for developing loco-regional recurrences based on pathological features. High risk group included those with involved surgical margins and extranodal spread. Low risk group included other adverse features such as T3/4 tumours, perineural invasion, lymphovascular space invasion, multiple nodal involvement and lower neck adenopathy. They advised for postop radiotherapy alone for patients with low-risk features and addition of concurrent chemotherapy for those with high-risk features. They also suggested postop radiotherapy dose of upto 63Gy for high-risk features and 57Gy for those with low-risk disease.

Perineural Invasion (PNI): There is no standardised definition of this entity, but the most accepted one is when tumour cells are present in any one of the three layers of the nerve sheath & when tumour cells are in close proximity to the nerve & involves more than one-third of circumference. Bur et al. identified an incidence of PNI in literature of between 3 and 52% [7]. Though many authors have not been able to assign prognostic significance to presence of PNI, some features such as multiple foci of PNI, involvement of large nerves (>1 mm) and higher maximum extent of PNI were associated with increased local failure and reduced disease specific survival.

In the systematic review collated data from 13 retrospective studies, they identified local recurrence rates of 4.4% - 22.9%, regional recurrence rates of 12.3% - 30.8% and 5-yr overall survival estimates of 48–89.6% in those patients with presence of PNI [7]. In two studies from this review, where neck dissection was conducted, regional failure rates were 12.3% and 17.6% which undermines the fact of ineffective salvage options after this. However, the authors suggest that in the absence of any prospective trial to assess the impact of PNI on loco-regional recurrence or survival, it would be imperative to discuss the options of treatment in detail in those patients where positive PNI is the only risk factor post radical surgery.

Depth of Invasion (DOI): Tumour thickness or depth of invasion has been consistently identified as a predictor for cervical lymph node metastasis in oral cavity cancers. Oral cavity cancers have occult nodal metastases of up to 40% in clinically negative neck, which is usually managed with elective nodal dissection as opposed to just observation. Huang et al. in their meta-analysis, have tried to address this aspect and have concluded that the optimal cut-off point for DOI is 4 mm, to consider for neck management [8].

In another study, Liao et al. followed-up patients who underwent surgery of early-stage oral cavity cancers(pT1-2 N0) to identify poor prognostic features and suggest for adjuvant therapy [9]. They found that poorly differentiated tumours and DOI of 4 mm and above were both independent poor prognostic factors, and when present together accounts for 2-yr regional failure of 42%. Hence, they suggest for the use of PORT in this subset of early OC cancers.

Bulbul et al. conducted a meta-analysis of 8 studies (1427 patients), which used frozen section (FS) evaluation to define margin status in early(T1/2N0) oral cavity cancers [10]. They compared positive/close FS margins which was cleared by further resection (R1 – R0), positive margin not cleared (R1) to those with negative margins upfront (R0). They found that patient with R1-R0 had poorer local recurrence free survival (LRFS) when compared to R0, regardless of clearance which was statistically significant. Furthermore, R1-R0 patients showed almost equal LRFS to

that of R1, though the trend to worse result was with R1 patients. They concluded that upfront positive/close margin was a marker of a locally aggressive disease regardless of re-resection & correction. They also indicated that there should be standardisation of the FS sampling method too.

3.2 Surgery followed by radiotherapy

The value of postoperative radiotherapy (PORT) for advanced head & neck cancers, was established in 1970's with few well researched studies [11]. Marcus et al. suggested doses for OC cancers of 6500Rads to achieve high local control and up to 7000rads for those with positive surgical margins [12].

Outcomes in locally advanced cancers are suboptimal with primary failure being loco-regional relapses. Hence combined modality treatments have been employed to counter this aspect as well as to ascertain if they added to overall survival patterns too. Lavaf et al. retrospectively analysed data from the Surveillance Epidemiology End Results (SEER) data base to collate results on 8795 lymph node positive cases of advanced head and neck cancers [13]. They found addition of adjuvant radiotherapy in these patients improved 5 yr. overall survival rates (43.2% vs. 33.4%; $p < 0.001$) and cause specific survival rates (50.9% vs. 42.1%).

Kao et al. did a similar SEER group analysis of advanced cancers of head and neck region and specifically tried to address benefit of RT with respect to various nodal stages and specific sites of disease [14]. Subset analysis found that all nodal stages, including N1 Disease, has improved survival with the addition of adjuvant RT. Also, in multivariate analysis, addition of RT in node positive oral cavity tumours improved overall survival [HR, 0.84; 95% CI, 0.73–0.98; $p = 0.025$], though it was not as significant as compared to the other head and neck sites.

Another population-based study done by Shrimeet al, tried to specifically address the benefit of PORT in early-stage primary oral cavity cancers (pT1/T2N1) with single ipsilateral node without extracapsular spread and being <3 cm in size [15]. For all patients, adjuvant RT was associated with superior 5 yr. overall survival [52.4% vs. 41.4%($p < .001$)]. Survival advantage was statistically significant in T2 cases, with a trend to significance in T1 cases. When individual sub-sites were analysed, PORT significantly improved survival in patients with cancers of oral tongue [52.3% vs. 37.9%($p = .002$)] and floor of mouth [39.9% vs. 17.7%($p = .003$)].

Addition of Chemotherapy to Radiation in post-op setting: Several retrospective and small prospective trials had showed improvement in outcomes of advanced head and neck cancers by adding chemotherapy (adjuvant or concurrent) to post-operative radiotherapy, especially in those with high-risk features such as inadequate margins, presence of extracapsular nodal spread and multiple involved nodes. Single institution trials by Bachaud and Smid, revealed improved loco-regional control rates and superior DFS/OS rates in combined modality group at a slightly higher rate of complications [16, 17].

Lacas & Pignon et al. (MACH-NC) in their updated meta-analysis of chemotherapy in head and neck cancer have studied the effect of concurrent chemotherapy use along with radiotherapy (upfront & post-surgery) [18]. Out of 107 studies (19,805 patients), 71 studies (10,680 patients) were on concomitant chemotherapy with a median follow up of 9.2 years. There was an absolute benefit in overall survival & event free survival of 6.5% & 5.8% at 5 years and 3.6% & 3.1% at 10 years with significant reduction in LRF rates [sub-HR = 0.71; $p < 0.0001$]. The study also showed that concurrent chemotherapy outcomes were better than induction chemotherapy and also that platin based chemotherapy gave maximum benefit, however there was no significant difference between weekly or three-weekly chemotherapy protocols in terms of outcomes or toxicity.

Bernier et al. (EORTC 22931) conducted a multi-institutional prospective study in 334 patients and found that addition of chemotherapy to adjuvant radiotherapy showed a benefit in 5 yr. PFS rates [47%(CMT) vs. 36%(RT)] and OS rates [53% (CMT) vs. 40%(RT)] & reduced the 5-year risk of death in combined modality from 43–27% [19]. Cooper et al.(RTOG 9501) did a similar study in 459 patients and showed significantly improved local/regional control as well as disease free survival (10% absolute benefit at 2 years) in the combined modality group after 45.9 months follow up [20].

As both these studies had few differences in their definition of high-risk disease, a pooled analysis was done to get some clarity and this concluded that outcomes for patients with ECE and/or involved surgical margins was significantly better with CMT [21]. Also, there was a trend to improved outcomes in patients with clinically enlarged level IV/V nodes with oral cavity/oropharyngeal primaries and perineural infiltration.

Three weekly high dose cisplatin (100 mg/m^2) is the standard systemic chemotherapy regimen given concurrently with radiation in high-risk oral cavity cancers. However, due to compliance issues low dose regimens have been applied in clinical practice without any prospective evidence to support the same. Szturz et al. conducted a meta-analysis to compare the standard three weekly regimen (100 mg/m^2; 3 doses) with the low dose weekly regimen $\leq$50 mg/m^2; $\geq$6 doses) [22]. Though there were no prospective comparative studies till date, 52 studies with 4209 patients were included and it showed no difference in the efficacy indices such as overall survival or response rates. In the definitive treatment setting, weekly regimen was more compliant and significantly less toxic with respect to myelosuppression, severe nausea/vomiting and nephrotoxicity. In the post-op setting, the two approaches were similar with the weekly arm causing more grade 3/4 dysphagia and weight loss.

JCOG1008 was a multi-institutional phase II/III non-inferiority trial comparing 3 weekly cisplatin with weekly schedule (40 mg/m^2) as a concurrent chemotherapy option in post-operative high-risk H&N cancers [23]. After enrolling 261 patients and a median follow up of 2.2 years, the trial was stopped as the non-inferiority criteria was reached. The 3-year OS (76.1% vs. 59.1%; HR of 0.69) &RFS (64.5% vs. 53%; HR of 0.71) was favouring the weekly arm as against the 3 weekly arm, confirming the non-inferiority of the lower dose weekly regimen.

3.3 Definitive radiotherapy (with or without chemotherapy)

There are no prospective trials which have directly compared primary surgery vs. primary radiotherapy in oral cavity cancers specifically. Two case series comparing these two modalities suggested a lower loco-regional control with primary radiotherapy compared to a surgical approach.

First was Studer et al., who assessed 58 consecutive oral cancer patients referred for either adjuvant or definitive radiotherapy [24]. They found local control rates were highest in the surgery followed by post-op IMRT group (92% LC at 2 yr), followed by patients treated with surgery alone or those receiving post-op 3DCRT (70–80% LC at 2 yr) and least in the definitive radiotherapy group treated with IMRT (40%LC at 2 yr) or 3DCRT (30% LC at 2 yr).

Murthy V. et al. studied 1180 oral cancer patients treated with PORT or definitive RT, by dividing them into 2 groups – Group 1 included gingiva-alveolar-buccal complex, lip and hard palate sites and group 2 included tongue and floor of mouth sites [25]. The 3 yr. LC, LRC and DFS for those treated with PORT was 74%, 65% % 60% respectively. And for those treated with definitive RT it was 34%, 31% & 30%

respectively. Also, they found Group 1 patients had significantly better LC, LRC and DFS than group 2 patients.

Cohen et al. did a retrospective review of 4 multi-institutional phase II trials dealing with primary chemo-radiotherapy for T4 oral cavity tumours [26]. In all 39 patients were assessed, 42% of them having bone involvement. Median dose of RT given was 74Gy. Five-year OS, PFS and LC were 56%, 51% & 75% respectively suggesting definitive chemoradiation to be a reasonable option in these tumours.

Hosni et al. retrospectively analysed all oral cancer patients (108 patients) treated with IMRT (with no surgical intervention) [27]. The cases had 63% cT3/T4 disease, 35% cN2/3 and 35% received concurrent chemotherapy. After a median follow up of 52 months, the 5-year local, regional and distant control rate was 78%, 92% & 90% respectively. The 5-year DFS, OS and CSS were 42%, 50% & 76% respectively justifying definitive non-surgical chemoradiation as a meaningful alternative in appropriately chosen cases.

Updated MACH-NC results showed the benefit of concomitant chemotherapy was due to its effect on deaths related to head and neck cancer (absolute benefit of 9.8% in 5 years) [18]. Addition of chemotherapy showed a significant reduction in loco-regional failure (sub-HR = 0.71; $p < 0.0001$) with a non-significant effect on distant failure.

The first Meta-Analysis of Radiotherapy in Head and neck cancer (MARCH) in 2005 and clearly showed an advantage of altered fractionation radiotherapy over conventional radiotherapy in terms of overall and progression free survival. The updated analysis of this study was conducted by Lacas et al. in 2017 by including 34 trials and 11,969 patients and extended the comparison to benefit of concomitant chemoradiotherapy over altered fractionation schemes [28]. They reiterated that altered fractionation was superior to conventional RT alone schemes in all disease indices and also that hyperfractionation schemes were better than accelerated RT plans especially when nodal disease was higher. The comparison between concomitant chemoradiation and altered fractionation revealed a clear benefit for CMT with an absolute benefit of 5.8% and 5.1% at 5 & 10 years respectively.

Combining altered fractionation RT schemes with chemotherapy in head and neck cancers have been studied in the following two trials. GORTEC 99–02 did a three-arm study comparing standard chemoradiation, accelerated radiotherapy-chemotherapy and very accelerated radiotherapy alone [29]. Progression free survival were 37.6%, 34.1% & 32.2% respectively with acute grade 3/4 toxicity of 76–84% in the altered fractionation arms. The RTOG 0129 trial did a direct head on comparison between standard fractionation chemoradiotherapy and accelerated chemotherapy-radiotherapy in 743 patients [30]. At a median follow up of 7.9 years, there were no differences in OS, PFS, LRF or DM rates between the two arms. Both these studies concluded that addition of chemotherapy to altered fractionation radiation schemes in locally advanced head and neck cancers provided no benefit in terms of outcomes.

3.4 Time factor in PORT

Two aspects of timing with regards to radiotherapy has been shown to be important for eventual outcomes in head and neck cancer management – overall treatment duration of radiotherapy as well as total time of both surgery and radiotherapy in CMT. Prolongation of both these indices seems to negatively impact the outcomes. Ang et al. conducted a multi-institutional study including 288 patients and found that in high-risk H&N cancers, there was a trend towards higher LRC and survival rates when PORT was delivered in 5 rather than 7 weeks [31]. Also, a

prolonged interval between surgery and PORT of >6 weeks or a total duration of surgery and PORT of >13 weeks significantly impacted outcomes negatively.

Huang et al. in their meta-analysis involving 46 studies dealing with this aspect found that in the combined analysis the rates of local recurrences were significantly higher among patients who received PORT more than 6 weeks after surgery (OR = 2.89; 95% CI) [32].

Fast tumour cell repopulation has been postulated as the reason behind why prolonging overall treatment time (OTT) can negatively impact local control and survival in cancers. González Ferreira et al. in their review of literature found prolongation of OTT resulted in an average loss of LRC ranging from 1 to 1.2%per day to 12–14% per day, requiring an average increase of 0.6–0.8Gy/day to compensate for it [33]. Also, they postulated that the lag period for the accelerated repopulation to be initiated was between 21 and 28 days.

Graboyes et al. conducted an institutional review to study this aspect and found that starting PORT > 6 weeks post-surgery resulted in decreased OS rates in both multivariate and propensity score-matched subsets [34]. They also found that increasing delay beyond 6 weeks resulted in small, progressive survival decrements [aHR 1.09, 1.10, 1.12 for 7–8 wks, 8–10 wks and >10wks respectively].

Zumer et al. did a retrospective analysis to identify the relationship between time before treatment intervention and tumour growth kinetics on treatment outcomes in those undergoing definitive radiotherapy with or without chemotherapy in 273 head and neck cancer patients [35]. There was no significant association between loco-regional control or survival indices and time to treatment intervention. They also found that the median tumour volume relative increase rate & tumour volume doubling time was 3.2%/day and 19 days respectively, but both had no impact on outcomes.

3.5 Dose & volumes considerations for radiation in oral cancers

As a principle in radiotherapy, at least for oral cavity squamous cell carcinomas, the dose needs to be delivered in the desired fractionated regimen without unnecessary interruptions and in the shortest time possible with no reduction in dose below that what is tolerated by late responding normal tissue.

This means that the total dose is important to prevent local recurrences and the factors that need to be kept in mind are [36].

- Dose
- Dose per fraction
- Overall treatment time
- Normal tissue toxicity

Radiotherapy in oral cavity tumours is associated with lot of acute as well as chronic toxicities which will be dealt separately. In order to minimise this, there are several steps taken and one of them is the use of highly conformal Intensity modulated radiotherapy (IMRT), and there is lot of data to support its use in head and neck cancers. Before starting radiation therapy all patients undergo a detailed examination by a dental surgeon and this is known as dental prophylaxis. The dental surgeon will assess the area of treatment and also estimate the dose that would probably be delivered to the surrounding bony structures as well as ascertain the status of the teeth and score it as per DMF (Decayed, Missing, Filled) index.

Based on this, treatment is advised and appropriately followed. By doing this exercise, the chances of osteoradionecrosis & soft tissue related long-term toxicities can be reduced or even eliminated. IMRT involves simulation and planning for which the most basic step is immobilisation by thermoplastic facemask attached to a base plate indexed to the treatment table. After the planning CT scan is done the volumes are outlined on them as per the guidelines [37].

3.5.1 For definitive radiation therapy

GTV or gross tumour volume is defined as the visible tumour along with the nodes which are abnormal. HRCTV [High Risk Clinical Target Volume] includes the margin around the GTV and also the first echelon group of nodes which will be level IB to level II. For LRCTV [Low Risk Clinical Target Volume] one would include level III, IV, supraclavicular and IA. Usually bilateral neck is to be treated, based on institutional protocol. PTV [Planning Target Volume] would be 5 mm around the CTV trimmed from skin, but based on institutional protocol, it could range from 3 mm to 10 mm (**Figures 5** and **6**).

3.5.2 For postop radiation therapy

HRCTV encompasses the area of the tumour bed [if features such as margin positivity, node positivity with extracapsular extension, peri-neural invasion and soft tissue extension is present]. IRCTV [Intermediate Risk Clinical Target Volume] would include the involved nodes without ECE & uninvolved adjacent nodal levels.

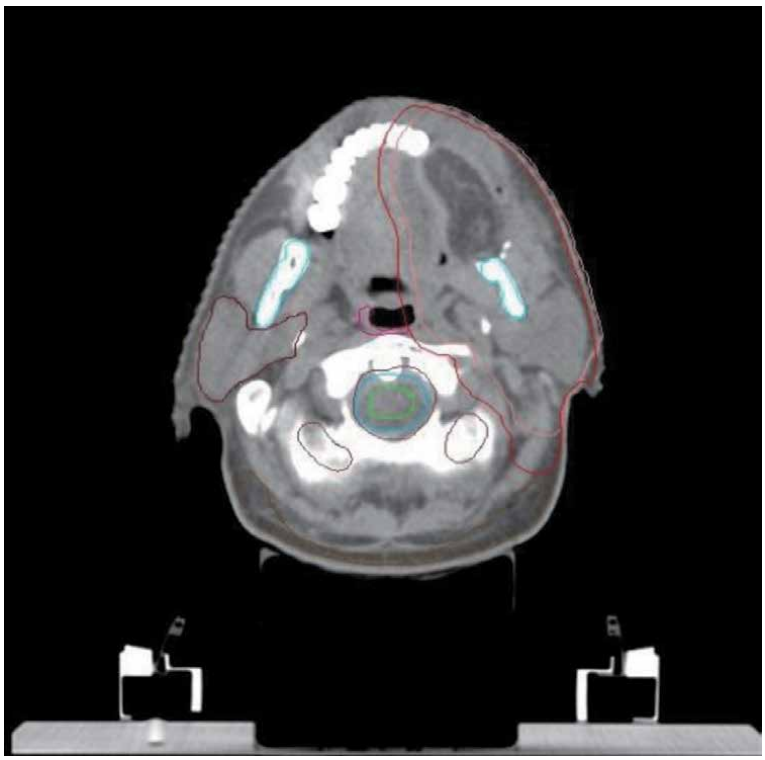


Figure 5.
Contouring of target volumes & organs at risk(OAR) for a case of carcinoma Oral tongue post surgery [pT3N1M0]; Orange – clinical target volume; Red – planning target volume; Cyan – mandible; brown – right parotid; green – spinal cord; pink - constrictor.

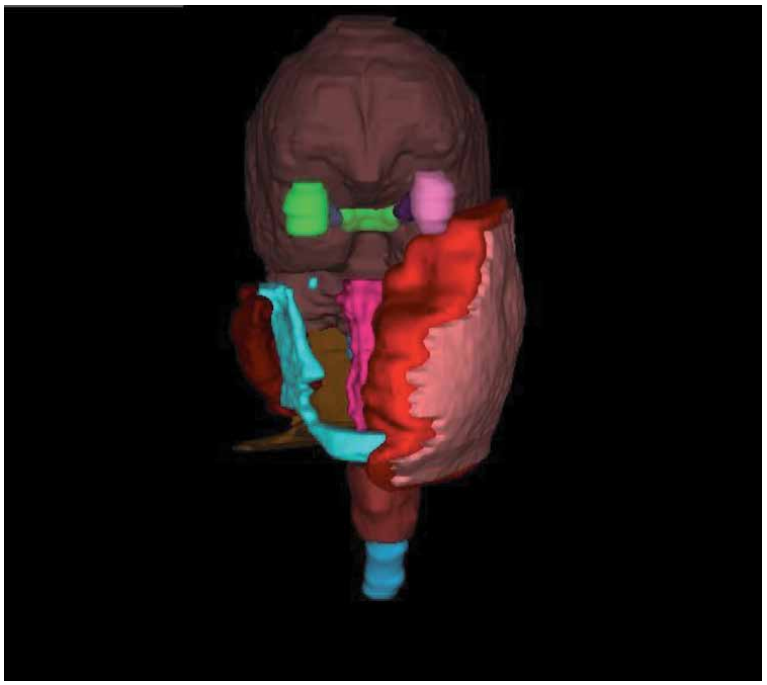


Figure 6.
3-dimensional projection of target & normal tissues contoured.

LRCTV would include rest of the nodal levels based on the risk stratification and bilateral neck is treated if risk is higher. PTV would be 5 mm around the CTV trimmed from skin, but based on institutional protocol, it could range from 3 mm to 10 mm (**Figure 5**).

As far as dose and dose per fraction is concerned there are multiple regimens and multiple doses available with their pros and cons. The following table depicts the usual practice with some insight on hypothesis (**Tables 3** and **4**).

3.6 Radiation related toxicity in oral cancer management

Radiotherapy is an essential part of multi-modality treatment for oral cancers. However, several uninvolved organs in the vicinity of these cancers like the skin, salivary glands, oral mucosa, masticatory apparatus, dentition and jaws receive significant doses of radiation during treatment. This could result in moderate to severe adverse effects during and after completion of treatment and also affect the patient's quality of life. The effects may be acute such as dermatitis, mucositis and hyposalivation or chronic and long-term such as xerostomia, radiation caries, trismus and osteoradionecrosis [38].

As many of these effects are dose-limiting, introduction of newer radiation techniques and schedules have minimised late effects to a large extent. Nutting et al. were able to demonstrate 50% reduction in subjective xerostomia rates with IMRT by keeping mean dose to contralateral parotid gland at 26Gy [39]. Parotid sparing IMRT was achieved by avoiding the contralateral parotid, upper parapharyngeal space as well as giving a tight constraint for the anterior oral cavity [36, 40].

A more problematic late effect is osteoradionecrosis, which is the process of bone and soft tissue necrosis, arising as a result of radiation induced hypocellularity, hypoxia and hypovascularity, resulting in a non-healing region [41]. As spontaneous ORN is dose dependent (>60Gy), IMRT is able to reduce the maximum

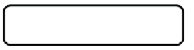
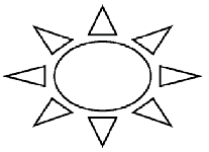
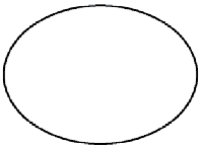
Post operative disease			
R1-Microscopic Disease 	R0 Resection 	R0 & N0 Disease	Volumes are applicable for conformal therapy. These volumes are subjective based on dose/fraction. Surgery to radiation interval should be </=6 weeks
NODE +, ECE/PNI + 	NODE +, No ECE/PNI 	ELECTIVE ONLY 	
RISK OF RELAPSE – >30%	RISK OF RELAPSE – 15–20%	RISK OF RELAPSE – 5–10%	
VOLUME – CTV-HR (PRIMARY)	VOLUME - CTVIR (PRIMARY) CTV IR(NODES)	VOLUME -CTVLR (NODES)	
DOSE – 66Gy–70Gy at 2Gy per fraction	DOSE – 60Gy at 2Gy per fraction	DOSE – 50Gy at 2Gy per fraction	

Table 3.
Dose & volume consideration for post-op adjuvant radiotherapy.

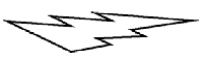
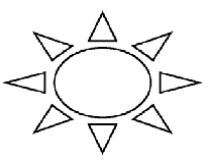
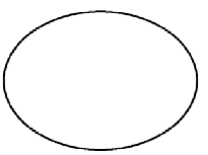
Definitive radiotherapy			
MACROSCOPIC DISEASE 	MARGIN AROUND THE GTV	NODE NEGATIVE, BUT DRAINAGE NODAL AREA	Volumes are applicable for conformal therapy. These volumes are subjective based on dose/fraction.
NODE + WITH ECE 	PRIMARY ECHELON 	ELECTIVE ONLY 	
VOLUME – GTV or CTVHR	VOLUME- CTVIR (PRIMARY) CTV IR(NODES)	VOLUME- CTVLR(NODES)	
DOSE – 66Gy–70Gy at 2Gy per fraction	DOSE – 60Gy at 2Gy per fraction	DOSE – 50Gy at 2Gy per fraction	

Table 4.
Dose & volume consideration for definitive radiotherapy.

dose received by the mandible as well as volume of mandible covered by 50-55Gy isodoses. The reported ORN rates in IMRT series is 5–6%.

Chen et al. found that IMRT also helped reduce dysphagia related complications in oral cancer patients undergoing radiotherapy [42]. Those receiving IMRT had significantly lesser moderate (grade 2) and severe(grade 3) dysphagia when compared to those receiving conventional radiotherapy (21% vs. 59%; p = 0.02) [42].

3.7 Targeted therapy, current bio-markers and future perspectives for oral cancers

Epidermal growth factor receptor (EGFR) regulates many cellular functions crucial for tumorigenesis. Huang et al. studied 160 oral cancer patients using immunohistochemistry for EGFR protein over-expression and fluorescence in situ hybridization for copy number [43]. EGFR overexpression was noted in 46.88% and 31.25% had increased gene copy numbers. They also found 100% concordance rate between EGFR gene amplification and protein overexpression. EGFR over-expression was associated with poor prognosis, both in terms of DFS and OS.

Cetuximab, an EGFR antibody has shown good results in head and neck cancers, in combination with definitive radiation [44]. The RTOG 0920 trial (Ongoing) is trying to address outcomes with addition of cetuximab to PORT in intermediate-risk oral cancers.

The development of PD-1/PD-L1 inhibitors (pembrolizumab & nivolumab) & other immune checkpoint inhibitors (ICI) has changed the systemic management of HNSCC [45, 46]. PD-L1 expression in pre-treatment biopsies have been associated with good prognosis. Preclinical data suggests synergy between anti-PD1 inhibitors and radiation, making it a potential therapeutic option for high-risk oral cancers in the future [47]. Several phase II studies addressing these agents in combination with standard therapy or as a neoadjuvant/adjuvant option are in the works [48].

Tumour mutational burden (TMB) as a biomarker of ICI response has shown mixed response in HNSCC, with KEYNOTE-012 trial showing a positive correlation while using a cut-off of ≥102 mutations per exome [49].

Other aspects being studied are tumour immune microenvironment and oral/gut microbiome as regulating mechanisms having implications for response of HNSCC to immune therapies. Oral microbiome has also shown an effect on toxicity profile of patients undergoing concurrent chemoradiation [50]. Cell therapy-based options such as the use of activated cytotoxic T-Lymphocytes (CTL's) to result in tumour cell death has also been attempted in HNSCC [51]. Chimeric antigen Receptor T cells are one such example being used in advanced oral cancers.

4. Brachytherapy

Brachytherapy is the delivery of radiation therapy using sealed sources placed within or close to the site to be treated. Oral cavity cancers with their ease of accessibility and better visibility had provided the best sites for use of this therapy for improved local control and outcomes in the past. With its ability to give high doses to tumour and very minimal dose to the surrounding tissues, brachytherapy could be considered the most ideal conformal therapy. However, in recent times the technological advancements associated with external beam therapy, improved imaging and surgical techniques and lack of appropriate knowledge or expertise in invasive implants has relegated brachytherapy to be used in very specific and not so common indications of these cancers. Modern brachytherapy too has evolved to

allow for acceptable dose and fractionation schedules, image guidance, dose optimization and better radiation protection mechanisms.

Common cancer sites where brachytherapy can be used in oral cavity are mobile tongue, lip, buccal mucosa, floor of mouth and palate [52, 53]. Indications for use of brachytherapy presently is

- Small localised T1 squamous cell cancers as primary treatment
- In combination of external beam radiotherapy as a boost modality
- As a re-irradiation modality in previously treated sites or development of second primaries

Disadvantages for brachytherapy are primarily due to lack of expertise and need for an initial learning curve which is usually lacking other than in bigger institutes, ease of modern conformal external radiotherapy techniques, competition with modern surgical techniques and concerns of radiation protection. Some relative contraindications to this procedure in oral cavity tumours would be compromised mouth opening, difficult naso-tracheal intubation & those having large defects requiring flap reconstructions in post-op setting.

Usually, the procedure followed is a single implant with multiple treatment fractions over nearly a week. Procedure is done under general anaesthesia with the help of nasotracheal intubation and dental separators to allow for proper visualisation. An interstitial implant is done following the principles of the Paris technique (**Figures 7** and **8).** A CT-scan based planning is done and the oncologist will delineate the tumour and organs at risk on the treatment planning system. Doses delivered are between 3-4Gy in 10–12 fractions delivered six hours apart over

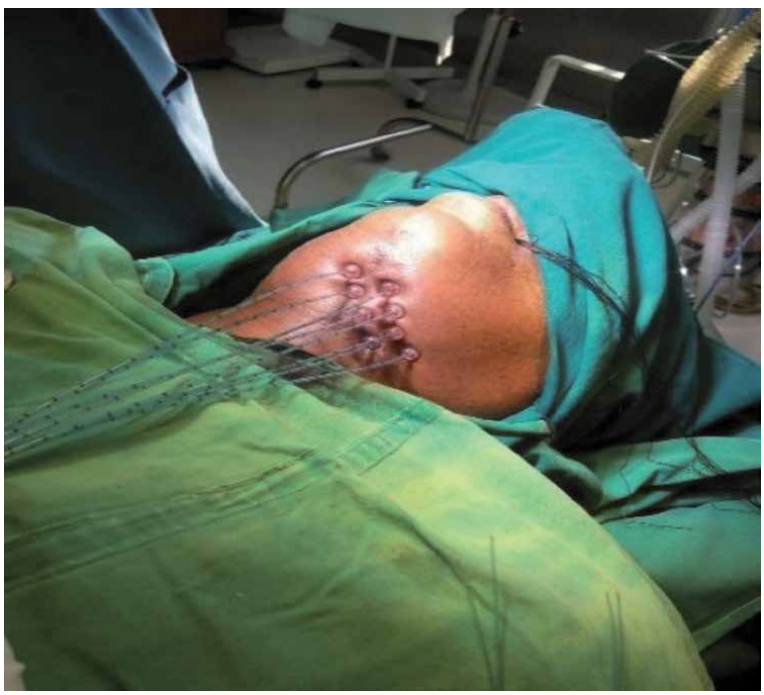


Figure 7.
Brachytherapy of tongue.

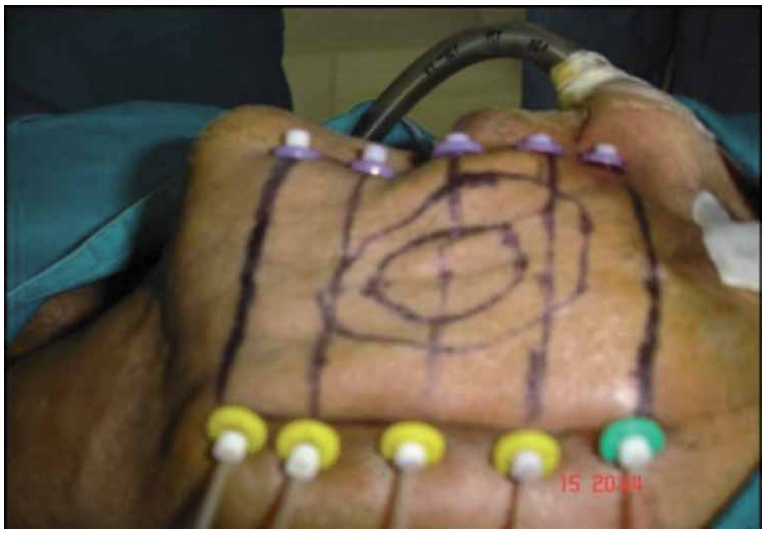


Figure 8.
Brachytherapy of buccal mucosa.

5–6 days in the primary treatment setting and 3-4Gy in 6–8 fractions over 3–4 days in the boost setting.

Acute complications of brachytherapy could be haemorrhage, infection, airway compromise & sialadenitis. Long term side effects could be soft tissue necrosis, telangiectasia and rarely osteoradionecrosis.

5. Conclusions

Oral cavity cancers are a diverse group of tumours which are known for its aggressive behaviour and higher chances of recurrences, which can lead to extremely difficult & cumbersome management decisions. This current review denotes the various available options for treatment for different categories of these tumours, to provide us a glimmer of hope to not only manage the disease well but also give good results in terms of locoregional control, long-term survival and at the same time ensuring cosmetically acceptable outcomes. As the primary focus of this chapter was on aspects of use of radiotherapy in oral cancers, the specific nuances on current evidence-based practice have been elaborated upon. Though most of the general details on use of external & internal radiation in oral cancers have been described, the detailed specific site-based & technique-based points have not been elaborated as they are beyond the scope of this chapter.

Available potential therapeutic options being currently investigated to ascertain possible benefit in oral cancers have also been described in this chapter. The future advances in use of radiation therapy should focus on recognising & refining the most appropriate indications for its application, strategic use of higher end technology like modulated arc therapy/stereotactic radiation & proton therapy, better amalgamation of radiation with newer concomitant systemic therapy agents as well as a well-rounded approach in inculcating genomic attributes of oral cancers in identifying the future standard of care for these tumours.

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References

[1] World Health Organization. Oral Health. 2020. Available online. https://gco.iarc.fr/today/data/factsheets/cancers/1-Lip-oral-cavity-fact-sheet.pdf. (Accessed on 11th July, 2021)

[2] Petrosyan V, Kane G, Ameerally P. Oral Cancer Treatment Through the Ages: Part 2. J Oral Maxillofac Surg. 2019 Jul;77(7):1484-1489. doi: 10.1016/j.joms.2019.01.024. Epub 2019 Jan 28. PMID: 30794811.

[3] Fred J. Laine, Wendy R.K. Smoker. Oral cavity: Anatomy and pathology, Seminars in Ultrasound, CT and MRI, Volume 16, Issue 6, 1995, Pages 527-545, https://doi.org/10.1016/S0887-2171(06)80024-7

[4] Cooper JS, Pajak TF, Forastiere A, Jacobs J, Fu KK, Ang KK, Laramore GE, Al-Sarraf M. Precisely defining high-risk operable head and neck tumors based on RTOG #83-03 and #88-24: Targets for postoperative radiochemotherapy? Head Neck 1998;20:588-94

[5] Langendijk JA, Slotman BJ, van der Waal I, Doornaert P, Berkof J, Leemans CR. Risk group definition by recursive partitioning analysis of patients with squamous cell head and neck carcinoma treated with surgery and postoperative radiotherapy. Cancer 2005;104:1408-17

[6] Expert Panel on Radiation Oncology-Head and Neck, Salama JK, Saba N, Quon H, Garg MK, Lawson J, McDonald MW, Ridge JA, Smith RV, Yeung AR, Yom SS, Beitler JJ. ACR appropriateness criteria® adjuvant therapy for resected squamous cell carcinoma of the head and neck. Oral Oncol 2011;47:554-9

[7] Bur AM, Lin A, Weinstein GS. Adjuvant radiotherapy for early head and neck squamous cell carcinoma with perineural invasion: a systematic review. Head Neck 2016;38 Suppl1: E2350-7

[8] Huang SH, Hwang D, Lockwood G, Goldstein DP, O'Sullivan B. Predictive value of tumor thickness for cervical lymph-node involvement in squamous cell carcinoma of the oral cavity: a meta-analysis of reported studies. Cancer. 2009;115:1489-1497.

[9] Liao CT, Lin CY, Fan KH, Wang HM, Ng SH, Lee LY, et al. Identification of a high-risk group among patients with oral cavity squamous cell carcinoma and pT1-2N0 disease. Int J Radiat Oncol Biol Phys. 2012;82:284-90.

[10] Bulbul MG, Tarabichi O, Sethi RK, Parikh AS, Varvares MA (2019) Does clearance of positive margins improve local control in oral cavity cancer? A meta-analysis. Otolaryngol Head Neck Surg 161:235–244.

[11] Fletcher GH, Evers WT. Radiotherapeutic management of surgical recurrences and postoperative residuals tumors of the head and neck. Radiology 1970:95:185-8. 57.

[12] Marcus RB Jr, Million RR, Cassissi NJ. Postoperative irradiation for squamous cell carcinomas of the head and neck: analysis of time-dose factors related to control above the clavicles. Int J Radiat Oncol Biol Phys 1979;5:1943-49

[13] Lavaf A, Genden EM, Cesaretti JA, Packer S, Kao J. Adjuvant radiotherapy improves overall survival for patients with lymph node-positive head and neck squamous cell carcinoma. Cancer 2008;112:535-43. 60.

[14] Kao J, Lavaf F, Teng MS, Huang D, Genden EM. Adjuvant radiotherapy and survival for patients with node-positive head and neck cancer: an analysis by primary site and nodal stage. Int J Radiat Oncol Biol Phys 2008;71:362-70

[15] Shrime MG, Gullane PJ, Dawson L, Kim J, Gilbert RW, Irish JC, Brown DH, Goldstein DP. The impact of adjuvant radiotherapy on survival in T1-2N1 squamous cell carcinoma of the oral cavity. Arch Otolaryngol Head Neck Surg2010;136:225-8

[16] Bachaud JM, Cohen-Jonathan E, Alzieu C, David JM, Serrano E, Daly-Schveitzer N. Combined postoperative radiotherapy and weekly cisplatininfusion for locally advanced head and neck carcinoma: final report ofa randomized trial. Int J Radiat Oncol Biol Phys. (1996) 36:999–1004.doi: 10.1016/S0360-3016(96)00430-0

[17] Smid L, Budihna M, Zakotnik B, Soba E, Strojan P, Fajdiga I, et al. Postoperative concomitant irradiation and chemotherapy with mitomycinC and bleomycin for advanced head-and-neck carcinoma. Int J RadiatOncolBiol Phys. (2003) 56:1055–62. doi: 10.1016/S0360-3016(03)00207-4

[18] Lacas B, Carmel A, Landais C et al. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 107 randomized trials and 19,805 patients, on behalf of MACH-NC Group. Radiother Oncol. 2021 Mar;156:281-293. doi: 10.1016/j.radonc.2021.01.013. Epub 2021 Jan 27. PMID: 33515668

[19] Bernier J, Domenge C, Ozsahin M, Matuszewska K, Lefèbvre JL, Greiner RH, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med. 2004;350:1945-52.

[20] Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med. 2004;350:1937-44

[21] Bernier J, Cooper JS, Pajak T, Van Glabbeke M, Bourhis J, Forastiere A, et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (# 22931) and RTOG (# 9501). Head Neck (2005) 27:843–50. doi: 10.1002/hed.20279

[22] Szturz P, Wouters K, Kiyota N, Tahara M, Prabhash K, Noronha V, Castro A, Licitra L, Adelstein D, Vermorken JB. Weekly Low-Dose Versus Three-Weekly High-Dose Cisplatin for Concurrent Chemoradiation in Locoregionally Advanced Non-Nasopharyngeal Head and Neck Cancer: A Systematic Review and Meta-Analysis of Aggregate Data. Oncologist. 2017 Sep;22(9):1056-1066. doi: 10.1634/theoncologist.2017-0015. Epub 2017 May 22. PMID: 28533474; PMCID: PMC5599190

[23] Naomi Kiyota, Makoto Tahara, Hirofumi Fujii, Tomoko Yamazaki, Hiroki Mitani, ShigemichiIwae, Yasushi Fujimoto, Yusuke Onozawa, NobuhiroHanai, Takenori Ogawa, Hiroki Hara, NobuyaMonden, Eiji Shimura, Shujiro Minami, Takashi Fujii, Kaoru Tanaka, Takeshi Kodaira, JunkiMizusawa, Kenichi Nakamura, Ryuichi Hayashi, and Head and Neck Cancer Study Group of Japan Clinical Oncology Group (JCOG-HNCSG). Phase II/III trial of post-operative chemoradiotherapy comparing 3-weekly cisplatin with weekly cisplatin in high-risk patients with squamous cell carcinoma of head and neck (JCOG1008).Journal of Clinical Oncology 2020 38:15_suppl, 6502-6502

[24] Studer G, Zwahlen RA, Graetz KW, Davis BJ, Glanzmann C. IMRT in oral cavity cancer. Radiat Oncol. 2007;2:16.

[25] Murthy V, Agarwal JP, Laskar SG, Gupta T, Budrukkar A, Pai P, et al. Analysis of prognostic factors in 1180

patients with oral cavity primary cancer treated with definitive or adjuvant radiotherapy. J Cancer Res Ther. 2010;6: 282-9.

[26] Cohen EE, Baru J, Huo D, Haraf DJ, Crowley M, Witt ME, Blair EA, Weichselbaum RR, Rosen F, Vokes EE, Stenson K. Efficacy and safety of treating T4 oral cavity tumors with primary chemoradiotherapy. Head Neck. 2009;31:1013-21

[27] Hosni, Ali et al. "Non-operative management for oral cavity carcinoma: Definitive radiation therapy as a potential alternative treatment approach." Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology vol. 154 (2021): 70-75. doi:10.1016/j.radonc.2020.08.013

[28] Lacas B, Bourhis J, Overgaard J, Zhang Q, Grégoire V, Nankivell M, Zackrisson B, Szutkowski Z, Suwiński R, Poulsen M, O'Sullivan B, Corvò R, Laskar SG, Fallai C, Yamazaki H, Dobrowsky W, Cho KH, Beadle B, Langendijk JA, Viegas CMP, Hay J, Lotayef M, Parmar MKB, Aupérin A, van Herpen C, Maingon P, Trotti AM, Grau C, Pignon JP, Blanchard P; MARCH Collaborative Group. Role of radiotherapy fractionation in head and neck cancers (MARCH): an updated meta-analysis. Lancet Oncol. 2017 Sep;18(9):1221-1237. doi: 10.1016/S1470-2045(17)30458-8. Epub 2017 Jul 27. Erratum in: Lancet Oncol. 2018 Apr;19(4):e184. PMID: 28757375; PMCID: PMC5737765.

[29] Bourhis J, Sire C, Graff P, Grégoire V, Maingon P, Calais G, Gery B, Martin L, Alfonsi M, Desprez P, Pignon T, Bardet E, Rives M, Geoffrois L, Daly- Schveitzer N, Sen S, Tuchais C, Dupuis O, Guerif S, Lapeyre M, Favrel V, Hamoir M, Lusinchi A, Temam S, Pinna A, Tao YG, Blanchard P, Aupérin A. Concomitant chemoradiotherapy versus acceleration of radiotherapy with or without concomitant chemotherapy in locally advanced head and neck carcinoma (GORTEC 99-02): an open-label phase 3 randomised trial. Lancet Oncol 2012;13: 145-53

[30] Nguyen-Tan PF, Zhang Q, Ang KK, Weber RS, Rosenthal DI, Soulieres D, Kim H, Silverman C, Raben A, Galloway TJ, Fortin A, Gore E, Westra WH, Chung CH, Jordan RC, Gillison ML, List M, Le QT. Randomized phase III trial to test accelerated versus standard fractionation in combination with concurrent cisplatin for head and neck carcinomas in the Radiation Therapy Oncology Group 0129 trial: long-term report of efficacy and toxicity. J Clin Oncol 2014;32:3858-66.

[31] Ang KK, Trotti A, Brown BW, Garden AS, Foote RL, Morrison WH, Geara FB, Klotch DW, Goepfert H, Peters LJ. Randomized trial addressing risk features and time factors of surgery plus radiotherapy in advanced headand-neck cancer. Int J Radiat Oncol Biol Phys 2001;51:571-8

[32] Huang J, Barbera L, Brouwers M, Browman G, Mackillop WJ. Does delay instarting treatment affect the outcomes of radiotherapy? a systematic review. J Clin Oncol 2003;21:555-63

[33] González Ferreira JA, JaénOlasolo J, Azinovic I, Jeremic B. Effect of radiotherapy delay in overall treatment time on local control andsurvival in head and neck cancer: review of the literature. Rep Pract Oncol Radiother2015;20:328-39.

[34] Graboyes, E.M., Garrett-Mayer, E., Ellis, M.A., Sharma, A.K., Wahlquist, A. E., Lentsch, E.J., Nussenbaum, B. and Day, T.A. (2017), Effect of time to initiation of postoperative radiation therapy on survival in surgically managed head and neck cancer. Cancer, 123: 4841-4850. https://doi.org/10.1002/cncr.30939

[35] Žumer, B., PoharPerme, M., Jereb, S. et al. Impact of delays in radiotherapy of head and neck cancer on outcome. Radiat Oncol **15,** 202 (2020). https://doi.org/10.1186/s13014-020-01645-w

[36] Daly ME, Le QT, Kozak MM, Maxim PG, Murphy JD, Hsu A, Loo BW Jr, Kaplan MJ, Fischbein NJ, Chang DT. Intensity-modulated radiotherapy for oral cavity squamous cell carcinoma: patterns of failure and predictors of local control. Int J Radiat Oncol Biol Phys. 2011 Aug 1;80(5):1412-22. doi: 10.1016/j.ijrobp.2010.04.031. Epub 2010 Aug 2. PMID: 20675073.

[37] Merlotti A, Alterio D, Vigna-Taglianti R, Muraglia A, Lastrucci L, Manzo R, Gambaro G, Caspiani O, Miccichè F, Deodato F, Pergolizzi S, Franco P, Corvò R, Russi EG, Sanguineti G; Italian Association of Radiation Oncology. Technical guidelines for head and neck cancer IMRT on behalf of the Italian association of radiation oncology - head and neck working group. Radiat Oncol. 2014 Dec 29;9:264. doi: 10.1186/s13014-014-0264-9. PMID: 25544268; PMCID: PMC4316652

[38] Basu T, Laskar SG, Gupta T, Budrukkar A, Murthy V, Agarwal JP. Toxicity with radiotherapy for oral cancers and its management: a practical approach. J Cancer Res Ther. 2012 Jan;8 Suppl1:S72-84. doi: 10.4103/0973-1482.92219. PMID: 22322736.

[39] Nutting CM, Morden JP, Harrington KJ, Urbano TG, Bhide SA, Clark C, et al. Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): A phase 3 multicentre randomised controlled trial. Lancet Oncol 2011;12:127-36

[40] Bhide SA, Ahmed M, Newbold K, Harrington KJ, Nutting CM. The role of intensity modulated radiotherapy in advanced oral cavity carcinoma. J Cancer Res Ther. 2012;8 Suppl1:S67-71.

[41] Eisbruch AH, Garden A, et al. Phase II Multi-institutional Study of IMRT for Oropharyngeal Cancer (RTOG 00-22): Int J Radiat Oncol Biol Phys 2006;66 Supplement.

[42] Chen WC, Hwang TZ, Wang WH, Lu CH, Chen CC, Chen CM, et al. Comparison between conventional and intensity-modulated post-operative radiotherapy for stage III and IV oral cavity cancer in terms of treatment results and toxicity. Oral Oncol. 2009; 45:505-10

[43] Huang SF, Cheng SD, Chien HT, Liao CT, Chen IH, Wang HM, et al. Relationship between epidermal growth factor receptor gene copy number and protein expression in oral cavity squamous cell carcinoma. Oral Oncol. 2012;48:67-72

[44] Bonner, J.A.; Harari, P.M.; Giralt, J.; Azarnia, N.; Shin, D.M.; Cohen, R.B.; Jones, C.U.; Sur, R.; Raben, D.; Jassem, J.; et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N. Engl. J. Med. 2006, 354, 567–578

[45] Muzaffar J, Bari S, Kirtane K, Chung CH. Recent Advances and Future Directions in Clinical Management of Head and Neck Squamous Cell Carcinoma. Cancers (Basel). 2021 Jan 18;13(2):338. doi: 10.3390/cancers13020338. PMID: 33477635; PMCID: PMC7831487.

[46] Herbst, R.S.; Soria, J.C.; Kowanetz, M.; Fine, G.D.; Hamid, O.; Gordon, M. S.; Sosman, J.A.; McDermott, D.F.; Powderly, J.D.; Gettinger, S.N.; et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014, 515, 563

[47] Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate nonredundantimmune mechanisms in

cancer. Nature (2015) 520:373–7.doi: 10.1038/nature14292

[48] Kirtane K, Rodriguez CP. Postoperative Combined Modality Treatment in High Risk Resected Locally Advanced Squamous Cell Carcinomas of the Head and Neck (HNSCC). Front Oncol. 2018 Dec 4;8: 588. doi: 10.3389/fonc.2018.00588. PMID: 30564559; PMCID: PMC6288294.

[49] Seiwert, T.Y.; Burtness, B.; Mehra, R.; Weiss, J.; Berger, R.; Eder, J.P.; Heath, K.; McClanahan, T.; Lunceford, J.; Gause, C.; et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): An open-label, multicentre, phase 1b trial. Lancet Oncol. 2016, 17, 956–965

[50] Pierce, C.M.; Hogue, S.; Paul, S.; Hong, B.Y.; da Silva, W.V.; Gomez, M. F.; Giuliano, A.R.; Caudell, J.J.; Weinstock, G.M. Abstract 3326: Mucositis, candidiasis, and associations with the oral microbiome in treatment naive patients with oropharyngeal cancer. Cancer Res. 2019, 79

[51] Ohtani, T.; Yamada, Y.; Furuhashi, A.; Ohmura, Y.; Nakamura, S.; Kato, H.; Yoshikawa, K.; Kazaoka, Y. Activated cytotoxic T-lymphocyte immunotherapy is effective for advanced oral and maxillofacial cancers. Int. J. Oncol. 2014, 45, 2051–2057

[52] Mazeron JJ, Ardiet JM, Haie-Méder C, Kovács G, Levendag P, Peiffert D, Polo A, Rovirosa A, Strnad V. GEC-ESTRO recommendations for brachytherapy for head and neck squamous cell carcinomas. Radiother Oncol 2009;91:150-6

[53] Nag S, Cano ER, Demanes DJ, Puthawala AA, Vikram B; American Brachytherapy Society. The American Brachytherapy Society recommendations for high-dose-rate brachytherapy for head-and-neck carcinoma. Int J Radiat Oncol Biol Phys 2001;50:1190-8

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Oral cancer is a common disease with a high incidence of morbidity and mortality. Despite technological advancements in diagnosis and treatment, the five-year survival rate is low. Researchers worldwide are attempting to identify new and novel methods for early diagnosis and better treatment of oral cancer to improve survival rate and quality of life post recovery. This book examines current concepts in oral cancer and emphasizes future perspectives for diagnosis and treatment of disease for better clinical outcomes and patient care.

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