Head and Neck Cancer Clinics
Series Editors: Rehan Kazi · Raghav C. Dwivedi

Peter A. Brennan
Tom Aldridge
Raghav C. Dwivedi *Editors*

Premalignant Conditions of the Oral Cavity

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Head and Neck Cancer Clinics

Series editors

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Head and Neck Cancer (HNC) is a major challenge to public health. Its management involves a multidisciplinary team approach, which varies depending on the subtle differences in the location of the tumour, stage and biology of disease and availability of resources. In the wake of rapidly evolving diagnostic technologies and management techniques, and advances in basic sciences related to HNC, it is important for both clinicians and basic scientists to be up-to-date in their knowledge of new diagnostic and management protocols. This series aims to cover the entire range of HNC-related issues through independent volumes on specific topics. Each volume focuses on a single topic relevant to the current practice of HNC, and contains comprehensive chapters written by experts in the field. The reviews in each volume provide vast information on key clinical advances and novel approaches to enable a better understanding of relevant aspects of HNC. Individual volumes present different perspectives and have the potential to serve as stand-alone reference guides. We believe these volumes will prove useful to the practice of head and neck surgery and oncology, and medical students, residents, clinicians and general practitioners seeking to develop their knowledge of HNC will benefit from them.

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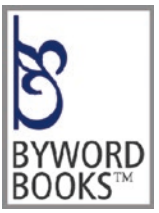
Peter A. Brennan • Tom Aldridge
Raghav C. Dwivedi
Editors

Premalignant Conditions of the Oral Cavity



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Preface

Oral cancer is a global healthcare problem with an increasing incidence year on year. While there have been many advances in the diagnosis, staging, treatment and reconstruction and rehabilitation following ablative surgery, the crude 5-year survival rates still remain at approximately 50%. Systemic chemotherapy using some of the newer monoclonal antibodies as well as the prompt treatment of early stage disease are associated with increased survival. New advances in surgery and radiotherapy including for example intensity-modulated radiotherapy (IMRT) are reducing post-treatment complications.

Oral squamous cell carcinoma (OSCC) is often related to smoking, alcohol consumption and other habits including betel or areca nut chewing. p16 has been more recently implicated in the aetiology of tumours of the oropharynx including tonsil and tongue base. Some OSCCs seem to arise de novo in clinically normal looking mucosa, while others occur following a premalignant disease. Therefore, the early recognition, diagnosis and management of these pre-cancerous diseases are crucial to improve survival and reduce morbidity for patients.

Research in both pre-malignant diseases and OSCC continues at a rapid pace, and it can be difficult to keep abreast of all developments particularly with some of the new and exciting molecular pathways and understanding of pathogenesis. In this unique new book, we have brought together respected experts and colleagues from around the world to provide a contemporary overview of the common premalignant conditions affecting the oral cavity. Following an overview which includes information on epidemiology and diagnosis, we have focused on the common diseases leading to potential malignant change in the oral cavity and their management. We have included cutting-edge research and developments across the specialties of oral medicine, oral pathology and OMFS.

With such a vast and ever-increasing subject, we apologise in advance for any omissions and would be grateful to receive feedback from readers with suggestions for the next edition of this book.

Portsmouth, UK — Peter A. Brennan
Portsmouth, UK — Tom Aldridge
Glasgow, UK — Raghav C. Dwivedi

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About the Editors

Peter A. Brennan, MD, FRCS, FRCSI, Hon FRCS Professor Peter Brennan is a Consultant Oral and Maxillofacial Surgeon at the Queen Alexandra Hospital, Portsmouth, UK, with an interest in head and neck oncology and reconstruction. He has a personal chair in surgery in recognition of his research and education profile, publishing over 530 papers to date as well as editing five major textbooks (including lead editor of the two-volume *Maxillofacial Surgery*) used successfully worldwide. He is lead editor for the new *Gray's Surgical Anatomy*—sister to the famous *Gray's Anatomy* itself.

Peter is committed to teaching and education at all levels and was previous Honorary Editor of the *British Journal of Oral and Maxillofacial Surgery*. In addition to reviewing for many reputed journals, he is the current editor of the *Journal of Oral Pathology and Medicine*—one of the most well-respected journals in this specialty area. Peter has research interests in oral cancer, neck anatomy, patient safety and human factors.

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Raghav Dwivedi, FRCS (Glas), PhD, FRCS (ORL-HNS) Raghav Dwivedi is a Consultant Head Neck Surgeon at the Queen Elizabeth University Hospital, Glasgow. He completed ENT and head neck surgery specialty training from the UK and India. He also completed head neck research fellowship at the Royal Marsden Hospital and the Institute of Cancer Research, London, and head neck surgical fellowships at Nottingham University Hospital, Cambridge University Hospitals, Portsmouth University Hospitals, and Imperial College, London. He holds an intercollegiate FRCS (ORL-HNS) from the Royal College of Surgeons of England; PhD from the Institute of Cancer Research, University of London, UK; and MS (ENT) from the King George's Medical University, India.

He is a dedicated head neck and ENT surgeon and has a unique blend of high-quality clinical and research experience. His areas of interest are minimally invasive head neck, thyroid and parathyroid surgery, HPV-related head neck cancers and outcome research. To date he has published 80 scientific papers in peer-reviewed indexed journals, 21 chapters (including one in the upcoming edition of Scott-Brown's *Otolaryngology: Head and Neck Surgery*) and edited one head neck surgery book. He has also served on the editorial board of six specialty journals and has been scientific reviewer for 35 peer-reviewed indexed journals including *BMJ*, *Cancer*, *Head and Neck*, *Oral Oncology* and *Surgical Oncology*.

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Chapter 1
Introduction

Peter A. Brennan and Tom Aldridge

We have invited leading experts from around the world to contribute to this book on the management of oral premalignancy. The book includes an up-to-date and comprehensive analysis of risk factors and systemic conditions that can lead to oral squamous cell carcinoma (OSCC) as well as a description of carcinogenesis at both molecular and genetic levels. Specific premalignant conditions are discussed, and detailed management strategies are provided. In the remaining chapters, current, interesting and useful information on the various premalignant conditions are included which we hope will enhance clinical practice and patient care.

In this introduction, we provide a brief overview of the epidemiology of oral premalignant disease and the potential impact that it has on our patients. We also give an overview on the structural and mucosal anatomy of the oral cavity and lips that makes this area such a challenging and complex location to manage.

Oral Premalignancy

Oral cavity cancer accounts for approximately 3% of all cancers. Most are oral squamous cell carcinoma (OSCC), and disappointingly the 5-year survival has not significantly improved over the last few decades, despite many advances in diagnosis, imaging and treatment modalities. Quality of life following oral cancer treatment has also improved with advances in free tissue transfer and targeted therapy including intensity-modulated radiotherapy (IMRT) which can spare adjacent structures such as the salivary glands and cervical spinal cord. Many OSCC tumours develop from premalignant conditions of the oral mucosa which are sometimes not detected or diagnosed before the cancer itself. Premalignant conditions have huge

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geographical, socioeconomic and population variation with an accepted prevalence of 1–5% and are most commonly found in the buccal mucosa, lower gingivae, tongue and floor of the mouth [1].

The World Health Organization originally recommended the terms 'precancerous lesions' and 'precancerous conditions'. A precancerous lesion is a morphologically altered tissue in which oral cancer is more likely to occur than in apparently normal counterpart. A precancerous condition is a generalised state associated with significantly increased risk of cancer. However, in 2005 these terms were simplified to 'potentially malignant disorders' to eliminate confusion from the previous used terminology, definitions and classifications of oral lesions with a predisposition to malignant transformation (Fig. 1.1) [2].

Oral precancerous lesions take many forms with leukoplakia, oral submucous fibrosis (OSMF) and oral erythroplakia being the most common (Fig. 1.2). There are other presentations of systemic conditions that can also be premalignant, such as xeroderma pigmentosum and Fanconi's anaemia. The link between carcinogenesis and immunodeficiency is also well known [3].

Although our knowledge is improving, the aetiology of premalignant conditions of oral mucosa is still incompletely understood [4]. There are well-recognised risk factors such as tobacco chewing, tobacco smoking, areca nut (for OSMF) and alcohol. While tobacco chewing is a major risk factor for oral leukoplakia, OSMF and erythroplakia, tobacco smoking may be a risk factor for oral leukoplakia. Alcohol drinking may increase the risk by 1.5-fold for oral leukoplakia, by twofold for OSMF, and threefold for erythroplakia.

The risk of malignant change in the external lip can occur with use of the above agents, but actinic damage following chronic sun exposure (UVA light) is the major risk factor associated with lower lip SCC (Fig. 1.3). The lower lip is at particular risk due to its reduced keratinised mucosa, reduced melanocyte number and orientation perpendicular to the sun and lack of protection from all but the widest brimmed hats.

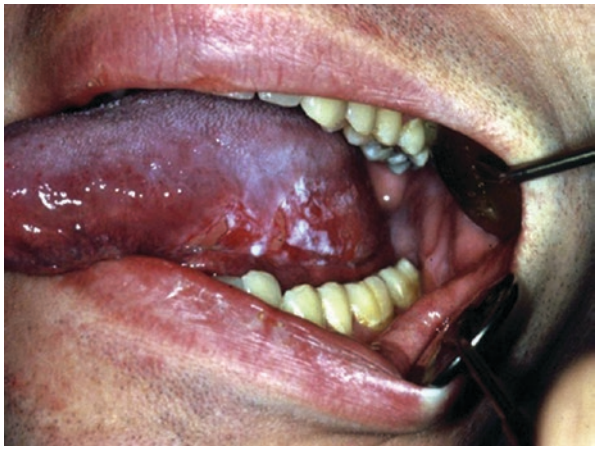

Fig. 1.1 Leukoplakia, left side of the tongue

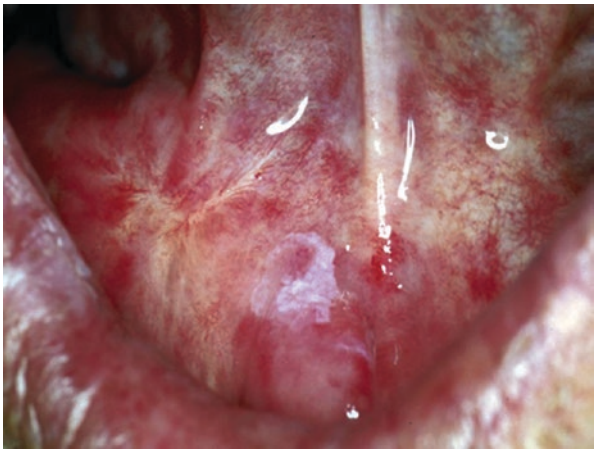

Fig. 1.2 Leukoplakia, floor of the mouth

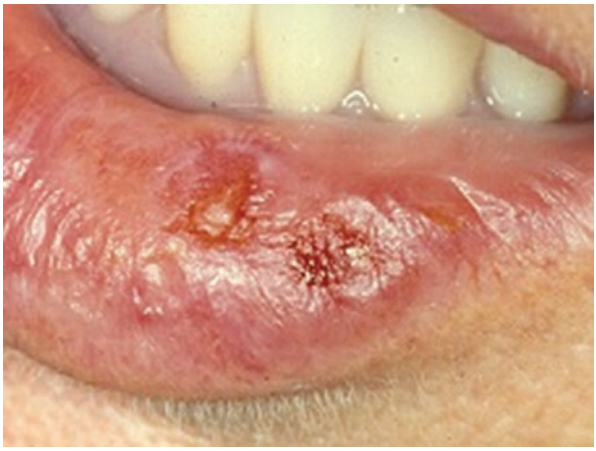

Fig. 1.3 Actinic keratosis, lower lip

For some strange reason, the minor salivary gland cancers well known in the upper lip are rarely seen in the lower lip, and almost all cancers are SCC from chronic sun exposure or tobacco use.

Embryology

The oral cavity develops from an ectoderm lined depression called the stomodeum. It is initially separated from the endoderm lined foregut by the transient buccopharyngeal membrane. Between the fourth and eighth week in utero, the frontal

prominence, together with the maxilla and mandible swellings of the first pharyngeal arch, develops to deepen the stomodeum. The pharyngeal arches each with their unique combinations of a nerve, muscle and cartilage go on to form the face and neck.

The first pharyngeal arch mesenchyme forms the maxilla which undergoes intramembranous ossification, and the mandible develops from intramembranous ossification of Meckel's cartilage. The muscles of mastication form from the first arch and hence receive motor innervation from the trigeminal nerve (fifth cranial nerve). The tongue develops concurrently with fusing of tissue from two lingual swellings and the tuberculum impar all derived from the first pharyngeal arch. These swellings form the anterior two thirds of the tongue and fuse with swelling from the second, third and fourth pharyngeal arches which themselves form the posterior one third. This explains the innervation of the posterior third innervation from the glossopharyngeal nerve.

Oral Mucosa

The oral cavity contains a complex variety of tissues from the hardest enamel to delicate salivary gland parenchyma. The oral cavity fuses with the skin at the vermillion and with the pharyngeal mucosa at the soft palate. The functions of the oral cavity are varied and require durability, special senses, protection and regeneration.

The oral mucosa itself consists of two layers with a surface stratified squamous epithelium and a deeper lamina propria. The histology of these components varies depending on the location. The epithelium is further divided into:

- Stratum basale
- Stratum spinosum
- Stratum granulosum
- Stratum corneum

The degree of keratinisation varies between location and function with keratinised mucosa being found on the attached gingivae, hard palate and dorsum of the tongue. Non-keratinised mucosa is found on the soft palate, inner lips, cheek, floor of the mouth and ventral tongue. These surfaces can become keratinised after periods of friction, for example, from poorly fitting denture or cheek biting (linea alba) or chemical irritation such as in 'smoker's palate' (nicotinic stomatitis). The classic sublingual keratosis found in smokers is also a well-known premalignant condition.

The oral mucosa can also be classified in terms of function, location or histology and can be divided into lining, masticatory and specialised mucosa.

Lining Mucosa

The oral surface of the lips, cheeks, floor of the mouth and ventral tongue are covered by a stratified non-keratinised epithelium. Deep to the epithelium lies the lamina propria where minor salivary glands are located. These glands become absent in the lips as the mucosa changes to keratinised skin at a junction called the vermillion border. Minor salivary gland tumours in the oral cavity are more likely to be malignant than benign from the outset, although the well-known pleomorphic adenoma ex carcinoma could be considered as a premalignant condition as it arises from a benign tumour.

Masticatory Mucosa

The attrition and friction that occurs on the masticatory mucosa requires a harder-wearing surface hence the need for keratinised epithelium. These surfaces include the gingivae and palate and are further strengthened by extensive interdigitation from the underlying lamina propria.

Specialised Mucosa

The epithelium of the tongue is complex. The thicker dorsal and lateral surfaces are keratinised, but not to the same degree as masticatory mucosa, and contain nerve endings for sensory and taste. The dorsal surface is unique with fungiform and circumvallate papillae which contain a lamina propria core.

Lips

The lip mucosa differs from the wet inner aspect, where minor salivary glands lubricate the surface, to the more exterior dry mucosa which lacks salivary glands and hence requiring licking to stay moist and to the outer dry mucosa which more resembles the skin.

The inner lip surfaces are covered with thick stratified squamous mucosa, whereas the dry outer surface is lightly keratinised. Long capillaries carry blood nearer to the surface hence the red appearance.

The lip is susceptible to oral and environmental carcinogens and is also a difficult surface to treat as it is not amenable to mouth rinses or many topical dermatological agents.

References

1. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37:1–10.
2. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45:317–23.
3. Thomas G, Hashibe M, Jacob BJ, Ramadas K, Mathew B, Sankaranarayanan R, Zhang ZF. Risk factors for multiple oral premalignant lesions. Int J Cancer. 2003;107:285–91.
4. Vlková B, Stanko P, Minárik G, Tóthová L, Szemes T, Bañasová L, Novotňáková D, Hodosy J, Celec P. Salivary markers of oxidative stress in patients with oral premalignant lesions. Arch Oral Biol. 2012;57:1651–6.

Chapter 2
The Molecular Basis of Carcinogenesis

Carolina Cavalieri Gomes, Marina Gonçalves Diniz, and Ricardo Santiago Gomez

In this chapter, we will discuss the molecular basis of carcinogenesis. *First understand, and then treat!* Better treatment options for cancer and preventive approaches for potentially malignant lesions can be achieved only if the pathobiology of the disease is well understood. We have witnessed a shift in the therapeutic approaches to cancer, from "universal" therapies applied to several different tumour types to tailored and personalized treatment. Each tumour/lesion is unique. As the understanding of malignant transformation and carcinogenesis requires knowledge of molecular and tumour biology, we aim to discuss carcinogenesis initially in a broader context before discussing the effects of carcinogens on the aetiology of potentially malignant oral lesions.

Starting from the Beginning: Useful Concepts

Carcinogenesis Theories and Field Cancerization in Oral Epithelium

How does cancer arise? Is it merely a result of the accumulation of mutations over time? Is cancer a disease of the cell, or is it a disease of the tissue and of cell signalling in the microenvironment? There are several theories that attempt to explain the process of carcinogenesis by incorporating evidence and developing models [1].

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Among these theories are coherent non-exclusive models of carcinogenesis that focus on the biological changes in the epithelium alone, whereas other models also take the changes in the stroma into account. By far, the most widely disseminated carcinogenesis theory is the “somatic mutation theory” (SMT), which is based on the assumption that cancer is derived from a single somatic cell that accumulates DNA mutations. The SMT focuses on molecular changes in the epithelium. On the other hand, the “tissue organization field theory” (TOFT) considers carcinogenesis as a problem of tissue organization, highlighting the importance of stroma in the process of carcinoma formation [2]. There are strengths and weaknesses in both models, and they are not mutually exclusive in some areas; however, the TOFT carcinogenesis model has gained acceptance recently, as more scientific evidence has strengthened the importance of the microenvironment in tumour formation, demonstrating that cancer is a disease of the tissue and not simply a cellular disease.

Regardless of the carcinogenesis model chosen to explain how normal cells become cancer cells, one needs to consider basic concepts in human molecular genetics, as clinical and histopathological morphological changes are accompanied by molecular changes in tissues. Slaughter proposed in 1953 the field cancerization process in oral stratified squamous epithelium, showing that clinically normal tissue surrounding oral squamous cell carcinoma (OSCC) already harboured histopathological changes [3]. Interestingly, once the structure of DNA was solved, the field cancerization concept evolved and was updated, and it became known that clinical and morphological normal tissues surrounding OSCC had already incorporated molecular changes [4] (Fig. 2.1). An understanding of this concept is fundamental for those studying/treating OSCC and oral leukoplakia. The field cancerization in oral mucosa can be as large as 7 cm [5], which means that by removing an oral leukoplakia lesion, one cannot remove all cells that have been molecularly altered. This knowledge is also fundamental when interpreting research studies whose

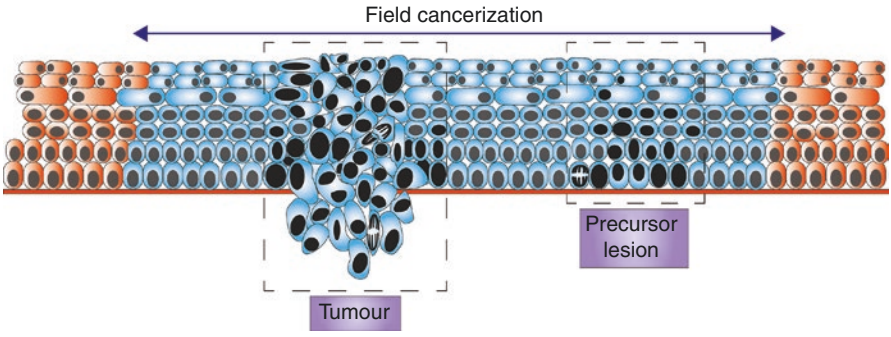

Fig. 2.1 Field cancerization. An area of epithelial cells harbouring molecular alterations (blue cells). A molecularly altered field can occur with normal histology, and in this figure we can observe a precursor lesion (oral leukoplakia) and an OSCC occurring in a same field cancerization

normal control reference tissues are “normal” tissues adjacent to the OSCC/oral leukoplakia.

Every pathology textbook describes the initiation and progression of cancer from a “clonal evolution” perspective. During clonal evolution, gradualism is assumed to occur, i.e. phenotypic features in cancers are believed to develop at a slow and continuous rate. According to clonal evolution, tumours are monoclonal, as they are derived from a single somatic cell, followed by the development of a neoplasm with cellular heterogeneity as a result of continued mutagenesis (we will discuss this topic in another section). When the tumour mass is established, clonal selection of the most well-adapted cells occurs, and the new, more fit clones rise to dominance and replace the entire population. This theory became the standard model of carcinogenesis and continues to spread, primarily because it is a simple and uncomplicated manner to explain a complex process. However, in this clonal evolution theory, even the definition of a “clone” is not unequivocal and straightforward and can be interpreted in more than one way [4]. Another caveat is that if cancers evolve linearly with time (gradualism), the malignant transformation of potentially malignant lesions, such as oral leukoplakia and Barrett’s oesophagus, should be predicted easily [6]. However, this phenomenon is not what happens in the clinic, as it is impossible to predict which “premalignant” lesions will evolve to become cancer.

Genetic progression models for oral leukoplakia have been proposed based on the somatic mutation carcinogenesis theory and on clonal evolution [5]. A monoclonal origin from OSCC associated with oral leukoplakia has been suggested, assuming that the carcinoma originated in the adjacent oral leukoplakia [7]. This hypothesis, however, is speculative, as retrospective studies using only the biopsy tissue from the excision of an OSCC lesion (including the adjacent oral dysplasia area) might not represent a true malignant transformation. OSCC is not always preceded by oral leukoplakia. To add a further layer of complexity to this subject, technological developments in genome analysis and mathematical and bioinformatics techniques have shown that the phenomena of punctuated and neutral evolution occurs during tumour evolution [6], and clonal evolution theory and gradualism fail to explain these findings. During the cancer evolutionary process, the genome is shaped not only by random mutations and non-random selection but also by random drift [4]. Both drift and selection change the frequency of alleles in a population, drift by random processes and selection based on *fitness*. Neutral evolution is defined as when selection is not operating and only the stochastic process of random mutations and drift occur. While random mutations and non-random selection have been the focus of several tumour evolution studies, random drift remains poorly understood, which does not allow for a complete understanding of how tumours evolve. A better understanding is yet to be obtained.

In the following sections, we will review briefly some basic concepts in human molecular biology. These definitions will help in following the discussions on cancer molecular pathogenesis.

DNA, RNA, Noncoding RNA, and Protein

The human genome is composed of DNA that contains approximately three billion base pairs distributed among 23 chromosome pairs (22 autosomal chromosomes and one sex chromosome). DNA molecules carry genetic information inside the cells and are composed of a double strand of linear polymers of nucleotides. DNA is packed inside the chromosomes in association with histone proteins, forming the nucleosomes. Each nucleosome consists of eight histone proteins around which DNA is wrapped [8], as shown in Fig. 2.2.

DNA is composed of the nucleotides adenine (A), cytosine (C), guanine (G), and thymine (T). It is organized into functional and physical units of heredity called genes. Genes have introns (regions which do not code for proteins) and exons (protein-coding sequences). The genetic DNA code is transcribed into mRNA, which is translated into proteins in that three nucleotides (codon) code for a specific amino acid in the protein (or are stop codons) [8].

Less than 2% of the human genome encodes proteins! Genetic sequencing of these protein-coding regions of the human genome is referred to as *whole-exome sequencing* (WES), and it is currently being used in biomedical research as well as

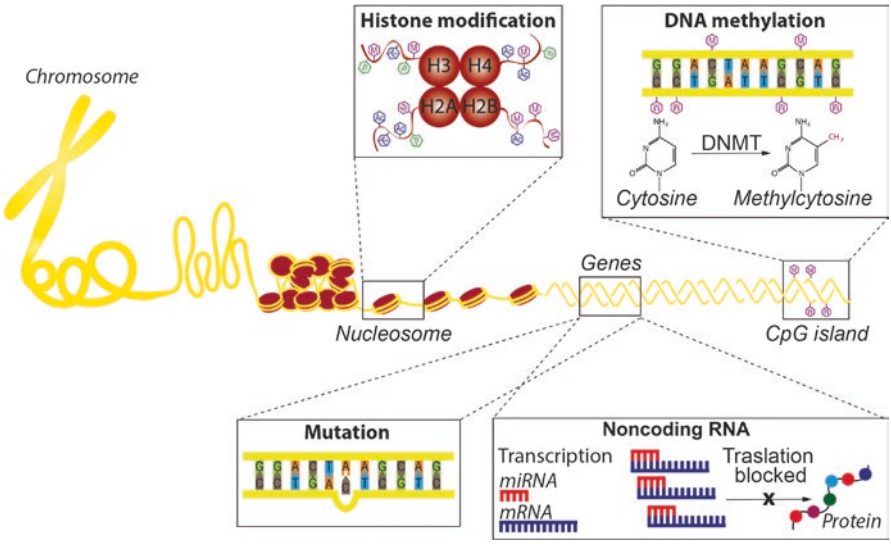

Fig. 2.2 DNA organization and carcinogenesis-related alterations. DNA is packaged in chromosomes forming complexes with histones. These complexes are the nucleosomes, and each nucleosome consists of eight histone proteins around which DNA is wrapped. Several alterations at nucleosome and nucleotide levels occur in carcinogenesis. The histone N-terminal tails modulate nucleosome structure and function and can suffer modifications, which include changes in their methylation and acetylation profiles. At nucleotide level, DNA mutations cause inactivation of tumour suppressor genes or activation of oncogenes. Gene expression levels can be altered by modifications in DNA methylation profiles (repressing transcription) or by ncRNA activity (repressing translation)

in the diagnosis of human diseases. Surprisingly, approximately 75% of the genome is transcribed into RNAs, including RNAs that have no protein-coding potential (noncoding RNAs) [9, 10]. Noncoding RNAs (ncRNAs) <200 nt are classified as small ncRNAs. Micro-RNAs (miRNAs) are a category of small ncRNAs. Conversely, ncRNAs >200 nt are classified as long ncRNAs (lncRNAs). While miRNAs are primarily involved in "silencing" gene expression (by targeting mRNAs) (Fig. 2.2), lncRNAs, which are more abundant than miRNAs in the human genome, exhibit a greater variety of functions in the regulation of gene expression [9].

miRNAs have been extensively studied in OSCC, and lncRNAs are in the process of being better characterized in such tumours [11, 12]. miRNA profiling in progressive and nonprogressive oral leukoplakias has shown that miR-21, miR-181b, and miR-345 increased expression in oral leukoplakias that progress to OSCC [13]. Additionally, higher expression levels of these miRNAs were found to be associated with cytological and histopathological parameters used to grade dysplasia, including an increased nuclear/cytoplasmic ratio and the presence of abnormally superficial mitosis [14]. LncRNA expression in oral premalignant lesions has been reported [15] but requires additional characterization and functional studies to better reveal the roles of such ncRNAs in the biology of these lesions.

Mutation and Genetic Variation

"There is no single sequence of the human genome." There are approximately three million sequence variations between any two unrelated persons, most of which do not have biological importance and do not contribute to physiological differences but do give rise to diversity between individuals.

Genetic variations that occur at a measurable frequency in the population are termed polymorphisms. A strict definition of a genetic polymorphism is variation present at a frequency ≥1% in the population. When a polymorphism is characterized by the substitution of a single nucleotide (e.g. the substitution of a C<T at a given position), it is defined as a single nucleotide polymorphism (SNP). Thousands of SNPs have been described, and there is a database of SNPs (and other short genetic variations) that can be accessed at https://www.ncbi.nlm.nih.gov/snp.

A mutation occurring in an exon (i.e. DNA that codes for proteins) can result in a change from one amino acid to another (missense mutation), a change that codes for a termination signal/stop (nonsense mutation), or no change in the amino acid (silent mutation). Mutations characterized by an insertion or deletion of one to a few nucleotides are called indels.

When DNA mutations are found in a given tumour, but not in peripheral blood/normal matching tissue, the mutation is considered a *somatic* mutation that originated in the tumour. However, if the mutation is also detected in normal constitutive DNA, it is classified as a *germline* mutation. An example of a germline mutation that predisposes individuals to cancer is the mutation in the *TP53* gene in Li-Fraumeni syndrome. However, the majority of tumours arise from somatic mutations and are

considered sporadic rather than familial tumours. Somatic mosaicism may occur, and a germline mutation cannot be detected in every constitutive normal cell; however, we will not discuss this topic in this review.

With the advances in next-generation sequencing (NGS) technology, the characterization of somatic genomic alterations in head and neck squamous cell carcinoma (HNSCC) is beginning to emerge. Recently, The Cancer Genome Atlas (TCGA) has profiled 279 cases of HNSCC by undertaking a comprehensive multiplatform characterization [16]. Similar to lung cancer and melanomas, HNSCC exhibits a high incidence of somatic mutations, which is consistent with its chronic exposure to mutagenic factors (tobacco smoking) [17]. Genes frequently mutated in HNSCC include *TP53*, *NOTCH1*, *HRAS*, *PIK3CA*, and *CDKN2A* [16]. *NOTCH1* gene mutations have been reported in a high proportion of oral leukoplakias and in OSCC, which raises the possibility of these mutations being important OSCC progression drivers [18].

Cell Cycle Differences Between Normal and Cancer Cells

Cell division occurs through sequential events that drive the progression from one cell cycle stage to the next, and it is altered in cancer cells [19]. The cell cycle is divided into two major phases, which are interphase and mitotic (M) phase. Interphase is subdivided into G1, S, and G2 phases. During G1, the cell grows and copies organelles; while in the S phase, the cell duplicates the DNA in the nucleus and in the centrosome. When the cell enters G2, it grows, synthetizes proteins and organelles, and prepares for mitosis. During the M phase, the cell separates its DNA and cytoplasm, leading to the formation of two cells.

Normal cells move through the cell cycle in a regulated manner, ensuring that they only divide when their DNA is not damaged and when there is room for more cells in the given tissue. The most important checkpoints that regulate the cell cycle are at the G1/S transition, the G2/M transition, and in the M phase. The cell cycle may be interrupted at any of these checkpoints so that the DNA can be repaired or that the cell can be eliminated by apoptosis.

Cyclins are one of the core cell cycle regulator proteins. Cyclins form complexes with cyclin-dependent kinases (CDKs), which in turn phosphorylate target proteins. There are several different cyclins, and the levels of each cyclin vary across the cell cycle, usually increasing only at the stage where they are required. Genetic mutations affecting cyclin or CDK genes can result in uncontrolled cell cycle progression. Cyclin D1, for example, is overexpressed in a variety of human cancers, including OSCC [20]. Conversely, there are CDK inhibitors that negatively control the cell cycle, including several different proteins such as p21, p16, p27, and p57. These proteins are frequently mutated or silenced by other mechanisms such as DNA methylation in human cancers. As CDKs play a central role in controlling cell cycle pathways, the development of therapeutic approaches to inhibit their kinase activity in cancer cells is currently in progress [21].

Alterations in the cell cycle include, but are not restricted to, genetic mutations (we will discuss this later in this chapter) and confer tumour cells with growth and survival advantages. While the normal cell cycle is regulated by proto-oncogenes, tumour suppressor genes, apoptosis genes, as well as DNA damage repair genes, in human neoplasia, these genes are usually dysregulated.

Oncogenes and Tumour Suppressor Genes

Oncogenes and tumour suppressor genes control cellular proliferation. An oncogene is a mutated form of a normal cellular gene referred to as a proto-oncogene. Proto-oncogenes are genes that positively regulate the cell cycle, and when they are over-activated by mutations, they are called oncogenes. This transformation of a proto-oncogene to an oncogene involves changes in protein amino acids, which can alter the protein structure. The mutations that convert proto-oncogenes to oncogenic alleles are named *activating mutations* to reflect "the gain of function". Additionally, proto-oncogene activation also can occur by gene amplification, in which extra gene copies are accumulated in the cell, resulting in extra protein production, or by chromosomal translocation (involving different mechanisms) [22].

Tumour suppressor genes are negative regulators of the cell cycle, and their functions are usually impaired in cancer. In contrast to proto-oncogene activating mutations, tumour suppressor genes usually harbour *loss-of-function* mutations with proteins that become functionally inactivated in cancer. Tumour suppressor genes normally control processes such as maintenance of genetic integrity, differentiation, cell-cell interactions, progression of the cell cycle, and apoptosis. Therefore, inactivation of tumour suppressor genes contributes to the disturbance of tissue homeostasis [23]. The most extensively studied tumour suppressor gene in human cancer is the *TP53* gene [24]. *TP53* prevents neoplastic transformation by temporarily or permanently activating the interruption of the cell cycle or by signalling cell death, and it is mutated in approximately half of all human cancer cases, including OSCC [16]. *TP53* is more frequently inactivated by small alterations, primarily by single nucleotide point mutations, and they occur at a higher frequency in hot spots that interfere with the functions of the encoded protein, which correspond to exons 5–8 of the gene.

Genetic Instability

Cancer cells commonly harbour defects in the mechanisms by which the genome is replicated and repaired and by which chromosomes are segregated during the cell cycle. These defects result in a higher rate of genetic alterations in cancer cells compared to normal cells and are less stable genetically than the surrounding normal tissue [25]. This genetic instability accelerates the occurrence of subsequent genetic

alterations; however, while genetic instability is a defect in a process, genetic alterations are stochastic events that do not necessarily indicate or cause genetic instability.

Genetic instability can be categorized into the following two major groups: instability at the nucleotide level and instability at the chromosomal level (chromosomal instability, CIN). Nucleotide-level instability includes deletions, insertions, and base substitution, while CIN refers to an increased rate of chromosome gains and losses, involving chromosomal missegregation due to mitotic errors [26]. A loss of specific chromosomal regions at constitutive heterozygous loci (loss of heterozygosity, LOH) that spans tumour suppressor genes has been reported to be a good predictor of malignant transformation of oral leukoplakia. Oral leukoplakias with LOH at chromosome regions 3p and/or 9p exhibited a markedly higher chance of malignant transformation compared to cases with 3p and 9p retention [27]. CIN involves cytogenetic changes that lead to changes in chromosome copy number, i.e. aneuploidy. Human cells contain 23 pairs of chromosomes and are diploid. A cell that has a number of chromosomes that is not a multiple of the haploid number is *aneuploid*. Aneuploid cells not only have a numerical abnormality but also commonly have chromosomal structural aberrations [26]. Aneuploidy occurs in a high proportion of solid human tumours, including OSCC [28]. In addition, as some OSCC arise in precursor lesions (potentially malignant oral disorders, including oral leukoplakia) and in preneoplastic epithelium, they can exhibit aneuploidy [29], and several studies have examined the possibility that aneuploidy indicates a risk of malignant transformation [30, 31]. Sperandio and co-workers [30] published a large series of DNA ploidy investigations in oral dysplasia, including 273 patients (32 with malignant transformation), for 5–15 years and demonstrated a positive predictive value for the malignant transformation by DNA aneuploidy of 38.5% [30]. In their study, the DNA ploidy status appeared to be correlated with epithelial dysplasia, and by combining both (ploidy status and dysplasia grading), the predictive value was higher than by using either technique alone. The utility of using DNA ploidy to predict the risk of oral dysplasia malignant transformations can vary according to the technique used, i.e. by flow or image cytometry [32].

While aneuploidy is a hallmark of several solid tumours, others do not show aneuploidy but rather exhibit defects in DNA repair. In a normal cell, DNA sequence errors arise as a result of mutagenic effects of environmental agents. In addition, errors caused by DNA polymerase arise during cell division (i.e. an endogenous form of mutagenesis). However, normal cells contain the machinery to repair these errors, as there are more than 100 known human DNA repair genes [33].

DNA repair pathways are classified into the following three functional categories: (1) direct reversal of DNA damage, (2) excision repair of DNA damage, and (3) DNA double-strand break repair. In the first pathway, a single enzyme repair system can restore the conformation of pyrimidines after UV light damage in a relative simple light-dependent reaction. The second pathway is composed of the following three different repair systems: base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) genes. BER proteins excise and replace

a single base and are commonly used to repair damage caused by insult to endogenous DNA (such as in response to oxidative DNA damage). NER excises oligonucleotides in response to genomic damage caused by UV exposure and involves at least 30 different proteins. MMR, the third excision repair system, preserves genomic integrity by acting in cases that involve inaccuracy in DNA replication. In the occurrence of a mutation during DNA replication, MMR recognizes and excises the mismatched nucleotide, resynthesizes DNA, and then ligates the broken strand. In addition, a direct reversal of DNA damage and excision repair of DNA damage can be repaired by a third pathway, which involves the repair of double-stranded DNA. This pathway uses a number of proteins to repair double-stranded DNA breaks (DSBs) that result from exogenous and endogenous agents, including ionizing radiation, chemical exposure, and somatic DNA recombination [33].

All of these mechanisms of DNA damage repair are interconnected and act cooperatively to maintain genome integrity. However, in cancer, these repair systems may be impaired. Mutations or loss of function of these genes may result in a reduced capacity for the correction of DNA errors, thereby predisposing the cell to genomic instability. If the functions of these genes are impaired, then the cell cannot repair the DNA, and programmed cell death can be triggered following the activation of apoptotic genes.

Evasion of Apoptosis

Tumour growth results not only from increased cell division, but it also depends on preventing cells from entering apoptosis. Neoplastic cells have the capacity to evade apoptosis by several mechanisms, enabling them to increase in number. These apoptosis-evasion mechanisms include the amplification of anti-apoptotic machinery, downregulation of the pro-apoptotic program, or both [34, 35]. There are several examples of altered regulation of genes that encode either the anti-apoptotic or pro-apoptotic Bcl-2 family in cancer. The *BCL-2* anti-apoptotic gene was first described because of its translocation in non-Hodgkin lymphomas, and it is also amplified in other tumour types [34]. Another mechanism that can lead to the overexpression of BCL-2 is the loss of micro-RNAs that repress BCL-2 gene expression, as observed in chronic lymphocytic leukaemia, in which micro-RNA 15 and 16 genes are deleted [10].

Immunotherapy and Immune Escape

The microenvironment is a critical regulator of tumour biology and can either inhibit or support malignant transformation and tumour development, growth, invasion, and metastasis. One important component of the tumour microenvironment is the immune system. Tumour cells express antigens that can mediate their

recognition by host CD8+ T cells and allow clinically detected tumours to evade antitumour immune responses.

Immunotherapy is an old concept, which has recently gained increased attention from the scientific community. These strategies are designed to alter the immune system, either by stimulating the patient's own immune system to attack cancer cells or by providing "immune system man-made components" such as proteins. Unfortunately, not all tumours respond to immunotherapy, and to increase the efficacy of immunotherapy, the immune escape mechanisms used by cancer cells must be overcome. Tumour cells can evade immune elimination by different mechanisms, such as the loss of antigenicity and/or the loss of immunogenicity, and by establishing an immunosuppressive microenvironment [36]. Immunotherapy is beginning to be explored in the oral cancer scenario, but the majority of novel immunotherapeutic strategies are currently investigational [37].

Epigenetics: Changes Beyond Genetic Sequence Changes

It is common to consider cancer a "genetic" disease. However, genetics and epigenetics cooperate in cancer development and progression. There is crosstalk between the genome and the epigenome. Genetic alterations of the epigenome contribute to cancer, and additionally, epigenetic processes can cause point mutations and disable DNA repair [38]. Epigenetics is defined as "heritable changes in gene expression that are not accompanied by changes in the DNA sequence". If we are not strict with the "heritability", noncoding RNAs can be considered epigenetic modifiers, and they have been discussed previously in this chapter. However, the most important epigenetic modifiers in cancer are DNA methylation, histone modification, and chromatin remodelling.

DNA methylation is classically associated with gene silencing, although other functions have recently been described. It occurs on cytosine, which is converted to 5-methylcytosine by the action of DNA methyltransferase (DNMT) enzymes (Fig. 2.2). Frequently, the altered C is adjacent to a G, and methylation is distributed in CpG sequences throughout the genome. CpGs are clustered in CpG islands, often at gene promoters (i.e. at the start of genes, where transcription machinery binds) (Fig. 2.2). CpG islands tend to be unmethylated, and when methylation occurs in CpG islands, it results in silencing of gene expression. DNA methylation can lead to gene silencing by different mechanisms that involve the physical impediment of transcriptional proteins binding to the gene and the indirect alteration of chromatin structure, forming heterochromatin. Heterochromatin is a compact and inactive form of chromatin. In cancers, the earliest epigenetic aberration found was a genome-wide hypomethylation [38]. Head and neck squamous cell carcinoma (HNSCC) exhibits global genomic

hypomethylation [39]. The degree of global methylation was associated with smoking history as well as with alcohol use and tumour stage in a large cohort of HNSCC samples [40].

In addition to DNA methylation, gene expression can be epigenetically modified by histone modifications, which include acetylation and methylation (Fig. 2.2). Most histone modifications occur on the N-terminal tails that protrude from the nucleosome (Fig. 2.2). Histone acetylation is universally correlated with gene activity and occurs at lysine (K) residues, and as it lacks mechanisms for mitotic heritability, it is considered a chromatin modification rather than an epigenetic modification. Histone methylation, on the other hand, can correlate either with transcriptional activity or with inactivity, and it occurs primarily at lysine (K) and arginine (A) residues [38]. Other histone modifications are less well characterized and include ubiquitination, phosphorylation, sumoylation, ADP-ribosylation, and citrullination. Very recently, impaired histone methylation (Histone H3 at K36, i.e. H3K36) was proposed to have a potential role in the development of a subset of HNSCC [41].

Interestingly, as a result of advances in next-generation sequencing, it was revealed that more than 50% of human cancers harbour mutations in chromatin organization enzymes. As tumour cells use epigenetic processes to escape from host immune responses and from chemotherapy as well, a growing number of studies are investigating drugs that target epigenomic alterations in cancer, including DNA methylation and histone modifications [42].

Intra-Tumour Heterogeneity

All of the aspects of tumour biology and molecular alterations and capabilities that have been described above must be understood in light of the "tumour heterogeneity" issue. Not all tumour cells share the same genetic and phenotypic traits, i.e. populations of tumour cells within the same tumour display remarkable variability. Intra-tumour heterogeneity is evident at the genetic and epigenetic levels as well as at the transcriptomic and proteomic levels [43, 44]. Intra-tumour heterogeneity is a phenomenon that has been known for several years, but it has recently gained more attention, as heterogeneity is a major obstacle to therapeutic success. Individual tumours may achieve resistance via several routes simultaneously, due to intra-tumour heterogeneity [45].

It is becoming increasingly evident that most, if not all, solid tumours exhibit evidence of intra-tumour heterogeneity. For some cancers, such as HNSCC and oesophageal and breast cancer, the degree of intra-tumour genetic heterogeneity is associated with a poor prognosis and a more negative clinical outcome. Of note, oral leukoplakia also shows intra-lesion heterogeneity with coexisting multiple "clones" [7].

Aetiological Factors for Oral Potentially Malignant Disorders (OPMDS) and Mechanisms of Carcinogenesis

Different aetiological factors are able to provoke genetic and epigenetic alterations in the genome (Fig. 2.3) [46]. Recent advances in sequencing technologies have deciphered the molecular signatures caused by mutagenic agents. For example, ultraviolet light (UV) and aflatoxin leave distinct patterns of DNA mutations in squamous cell carcinomas and hepatocellular carcinomas, respectively. Below, we review the most important aetiological factors currently associated with the occurrence of OSCC and oral potentially malignant disorder (OPMD).

Tobacco Smoking

Oral leukoplakia is the main oral potentially malignant disorder (OPMD). Although the association of oral leukoplakia with smoking and alcohol is well accepted in the literature, there is a lack of well-designed studies that deeply investigate this issue [47]. Systematic reviews are hampered by the heterogeneity of the studies and by changes in the oral leukoplakia concept and definition with time. The association between tobacco smoking and oral leukoplakia is based primarily on observational studies that report the disappearance of some lesions following the cessation of tobacco smoking. A Cochrane review discussed the lack of trials evaluating smoking cessation and the evolution of disease in patients [48].

There are approximately 20 substances in cigarette smoke that produce carcinogenic effects. The most important of these substances are nitrosamines, polycyclic aromatic hydrocarbons, aromatic amines, and aldehydes. Nicotine in tobacco has no

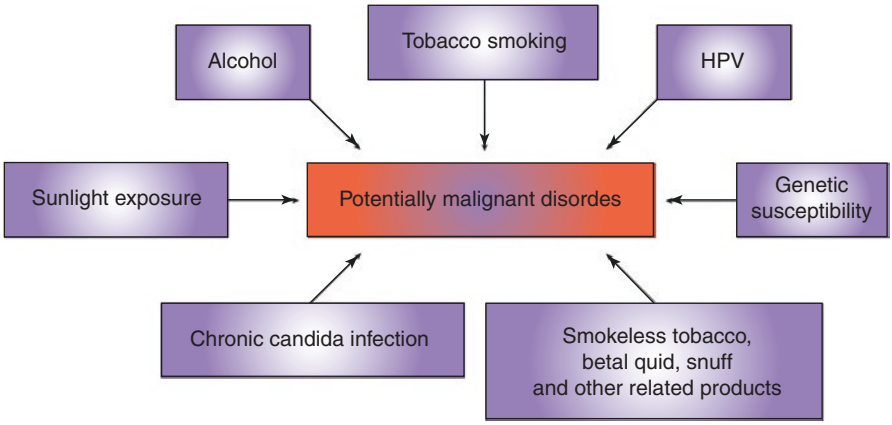

Fig. 2.3 Aetiological factors of oral potentially malignant disorders

carcinogenic effect, but it is a highly addictive substance. In general, tobacco products are carcinogenic only after metabolic activation; however, host enzymes can detoxify them.

Nitrosamines are found in smoked and smokeless tobacco, and their metabolites can covalently bind to DNA, forming DNA adducts that can promote mutations [49]. However, the carcinogenic effect of nitrosamines requires metabolic activation. In addition to forming DNA adducts, nitrosamines generate hydroxyl radicals or other reactive oxygen species that can damage DNA and cause single-strand breaks. Benzopyrene, a polycyclic aromatic product, and aromatic amines can cause mutations in *TP53* and the formation of different DNA adducts. Acrolein, an aldehyde present in tobacco, is an active carcinogenic product and is associated with mutations in *TP53*. Acrolein adducts inhibit nucleotide excision repair enzymes, which, as discussed earlier in this chapter, is an important mechanism for the repair of DNA damage caused by tobacco products.

It is interesting that tissues directly exposed to tobacco products, as well as those not directly in contact with them, show elevated levels of DNA adducts in smokers. A recent study demonstrated a predominance of T>C and C>T mutations in oral cancer cells in smokers, and these alterations were correlated with age at the time of diagnosis of the disease [50].

Tobacco products may also cause methylation of tumour suppressor genes, induction of oxidative stress, and inflammatory reactions. Oral cancer cells in smokers contain more hypomethylated and hypermethylated genes than non-smokers, indicating a change in the normal methylation pattern. Recent studies have also demonstrated altered expression of miRNAs in tobacco-related neoplasias.

Smokeless Tobacco, Betel Quid, Snuff, and Other Related Products

Smokeless tobacco is a term used to define the consumption of tobacco without burning, and it is a risk factor for OPMD. There are a variety of smokeless tobacco products that can be chewed, sucked on, or sniffed. They also can be used together with other ingredients such as areca nut, lime, spices, and ash. Tobacco is sometimes boiled or burned for consumption. Smokeless tobacco can cause the formation of DNA adducts and the production of reactive oxygen species, which can cause mutations in several genes, including *HRAS*, *KRAS*, *NRAS*, and *TP53* [51]. Smokeless tobacco also may cause disruption of the cell cycle by the hypermethylation of tumour suppressor genes [52].

Betel-related products for chewing or betel quid usually include betel leaf, lime, tobacco, and betel nuts. Betel quid has two basic carcinogenic actions in the oral mucosa. The first is the cytotoxic and mutagenic effect of its components (arecoline, alkaloids and polyphenols) on epithelial cells, while the second is associated with induced fibrosis, which reduces the oxygen supply to the epithelial cells.

Chewing betel quid is strongly associated with the development of oral submucous fibrosis, which is an important OPMD that occurs specially in South Asia [53]. The mechanism by which betel quid produces submucous fibrosis in oral tissues involves the action of its different components. This mechanism mainly involves suppression of endothelial cell proliferation; generation of reactive oxygen species; activation of NF-kB, JNK, and p38 pathways; production of connective tissue growth factors; and upregulation of TGF-b. These alterations cause DNA damage, progressive accumulation of collagen, and cross-linking of collagen fibres, which renders them less susceptible to breakdown. These effects explain the fibrotic nature of the disease, and the loss of vascularity leads to atrophy of the epithelium.

Recent studies have also suggested that areca nut compounds are involved in the epithelial-mesenchymal transition [54]. The epithelium-mesenchymal transition phenomenon has an important role in differentiation, migration, and invasion of keratinocytes, and it has been implicated in the malignant transformation of oral submucous fibrosis. Other molecular changes induced by betel components include the overexpression of CAIX, a hypoxia-inducible enzyme overexpressed by cancer cells, and the decreased expression of tumour suppressor genes, such as *PTEN* and *BRCA* protein-related genes.

Alcohol

The role of alcohol in OSCC is more clearly established than in the development of oral leukoplakia. A prospective study reported by Maserejian et al. (2006) [55] demonstrated that alcohol consumption is an independent risk factor for oral leukoplakia; however, this finding was not confirmed definitively by other reports. The independent risk effect of low/moderated alcohol consumption is unclear, considering the different types of beverages available.

Alcohol dehydrogenase (ADH) catalyses the oxidation of alcohol to acetaldehyde, which is the major metabolite of alcohol [56]. This process occurs in the cytoplasm. In chronic alcohol consumption, the CYP2E1 enzyme is utilized and results in acetaldehyde formation in peroxisomes. Acetaldehydes are very toxic and affect DNA synthesis and repair. Because of its electrophilic nature, acetaldehyde can bind and form adducts with proteins, lipids, and DNA, which impairs their functions and promotes DNA damage and mutation. The carcinogenic effect of alcohol is also mediated by increased oxidative stress, release of inflammatory cytokines, impairment of retinoid metabolism, and inhibition of DNA methylation.

As acetaldehyde is toxic and can cause health problems, it needs to be oxidized to acetate by the enzyme aldehyde dehydrogenase [56]. As the acetate formed is unstable, it breaks down spontaneously to CO_2 and water. Genetic factors can influence the propensity for the accumulation of acetaldehyde. SNPs in the alcohol

dehydrogenase and aldehyde dehydrogenase genes can result in the toxic accumulation of acetaldehyde, thereby enhancing its procarcinogenic effect.

HPVs

Human papillomaviruses (HPVs) are small double-stranded DNA viruses, and their family consists of more than 130 types, including high-risk and low-risk types [57]. Among the many high-risk HPVs, HPV-16 is the most common, and it accounts for approximately 90% of HPV-positive carcinomas of the oropharynx [58]. These viruses are sexually transmitted primarily through direct contact, and the majority of infections clear spontaneously within 24 h; however, this does not necessarily create immunity. HPV-positive head and neck cancers, when compared to HPV-negative counterparts, affect younger patients and are less likely to be associated with risk factors such as smoking and alcohol [59]. While less than 5% of non-oropharyngeal head and neck cancers are caused by HPV infection, greater than 70% of oropharyngeal cancers are related to this virus [60, 61]. A recent meta-analysis suggested that HPV16 is a significant independent risk factor for oral leukoplakia [58].

Recent studies have demonstrated molecular mechanisms in which HPVs induce carcinogenesis. E6 and E7 HPV proteins function as the dominant oncoproteins of high-risk HPVs, and they inactivate the tumour suppressor proteins p53 and pRB, respectively [57]. *TP53* is the "guardian of the genome", and its malfunction in most cancers is the result of DNA mutation. In HPV-associated cancers, the E6 oncoprotein degrades the wild-type p53 protein and leads to chromosomal instability in a manner similar to of DNA mutations. HPV E7 protein inactivates pRB, which releases E2F and promotes the transition from the G1 to the S phase of the cell cycle by transcription of the cyclins E and A. The disruption of pRB causes overexpression of p16, which explains why the overexpression of p16 is one of the markers of the infection used in immunohistochemistry. The immunohistochemical study of p16 protein in conjunction with in situ *hybridization* is the gold standard for the diagnosis of HPV-associated cancer. HPV-negative oropharyngeal cancer is associated with approximately twofold more mutations than the HPV-associated counterpart. HPV-positive head and neck cancer has an improved prognosis; however, its precursor lesion in the oropharynx has not yet been identified.

Chronic Candida Infection

Despite the extent of the oral presence of *Candida albicans* being higher in patients with OSCC or oral leukoplakia, the role of this microorganism in oral carcinogenesis is not well established [62, 63]. *C. albicans* produces nitrosamines that are

important carcinogenic compounds. Nitrosamines, after metabolic activation by cytochrome P450 enzymes, induce alkylating DNA damage by formation of the highly reactive diazonium ion, which leads to mutations in DNA. Point mutations can activate specific oncogenes or suppress tumour suppressor genes, as discussed earlier in this chapter. Additional potential mechanisms by which *Candida* spp. may promote oral carcinogenesis include the inflammatory reaction associated with infection and the metabolism of ethanol with the consequent production of acetaldehyde, a potential carcinogenic compound.

Sunlight Exposure

Long-term exposure to sunlight is the major aetiological factor of cancer in the lower lip. Actinic cheilitis is an OPMD of the lower lip, and it can progress to squamous cell carcinoma. There are three types of ultraviolet radiation (UV) that can damage the genome: UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm). UVB and UVC can produce DNA photoproducts, including pyrimidine photoproducts. These photo lesions can cause UV signature mutations (C>T transitions and CC>TT tandem double mutations), leading to upregulation and downregulation of signal transduction pathways and cell cycle dysregulation [64]. The CC>TT transition in *TP53* has been reported in lip squamous cell carcinomas as well as in actinic cheilitis. Additional effects of UV include the depletion of antioxidant defences and the induction of local immunosuppression. Nucleotide excision repair enzymes are able to repair DNA by removing UV-induced photo lesions [65]. Therefore, nucleotide excision repair enzymes counteract the formation of mutations and the development of skin/lower lip cancers.

Genetic Susceptibility to OPMD

Although the risks of lifestyle exposures to environmental carcinogens are associated with the development of premalignant lesions in the oral mucosa, genetic susceptibility helps to explain interindividual or interpopulation variations. Most studies are dedicated to the investigation of genetic risk factors for the development of oral cancers, and few of them are focused on OPMD.

A variation in a single nucleotide that occurs at a specific position in the genome is known as a single nucleotide polymorphism (SNP) (reviewed earlier in this chapter), and it can change the amino acid sequence of a protein. This change can affect the protein's function and its ability to metabolize carcinogens or its capacity to repair DNA damage caused by a carcinogenic substance.

Carcinogenic compounds related to oral cancer can be activated or degraded by a certain group of enzymes known as xenobiotic metabolizing enzymes (XMEs).

The metabolism of tobacco products, for example, involves oxygenation by P450 enzymes in cytochromes and conjugation by glutathione-*S*-transferase. Many XME SNPs can influence the individual's biological response to carcinogens. Because of the mutagenic effect of acetaldehyde, SNPs in the enzymes involved in alcohol metabolism (alcohol dehydrogenase and aldehyde dehydrogenase) are also related to the risk of developing oral cancer [66].

Genotype variations associated with increased susceptibility to the development of OSCC also include genes related to inflammation, stabilization of the genome, regulation of cell proliferation, apoptosis, and tumour survival [66]. Therefore, SNPs can partly explain the genetic susceptibility to human diseases, including the development of oral cancers and potentially malignant lesions. The investigation of SNPs may be helpful in identifying patients who are affected by OPMDs that may present an increased risk for malignant transformation.

Conclusion

Histopathological examination is not sufficient to accurately predict the malignant potential of OPMD. Despite being the gold standard method for assessing the grade of dysplasia, mild dysplastic lesions may progress to OSCC, while lesions with higher dysplastic features may not suffer malignant changes. For example, only approximately 5% of oral leukoplakias progress and transform into OSCC. However, the challenge is to identify which lesions are at risk and which lesions will never progress. Therefore, several studies have attempted to identify molecular changes associated with the malignant progression of oral leukoplakia.

As cancer development and progression indicate instability in the genome, this feature has been studied in OPMD, and chromosomal instability was reported to be a reliable method for the assessment of premalignant lesions of the oral mucosa at risk for transforming into cancer [67]. Other malignant transformation markers are beginning to be identified. LOH patterns were shown to be able to predict oral leukoplakia lesions at risk for malignant transformation. Epigenetic changes are also relevant to malignant progression, and hypermethylation of p16 is apparently associated with a higher potential of oral leukoplakia malignant transformation [68]. Specific miRNAs were demonstrated to be overexpressed in oral leukoplakia that progressed to oral cancer, and some cytological and histopathological parameters used to grade dysplasia are associated with altered expression of miRNA [13, 14].

There are several layers of complexity that surround the oral malignant transformation issue. One needs to keep in mind that the individual interacts with the environment (and potential carcinogen sources) as the epithelium interacts with the microenvironment (extracellular matrix, blood vessels, fibroblasts, immune cells, etc.) and the genome interacts with the epigenome. In addition, the utility of molecular and histopathological profiling is limited by intra-lesional heterogeneity, which may in part explain the discordant results in the literature.

References

1. Vineis P, Schatzkin A, Potter JD. Models of carcinogenesis: an overview. Carcinogenesis. 2010;31:1703–9.
2. Sonnenschein C, Soto AM. Theories of carcinogenesis: an emerging perspective. Semin Cancer Biol. 2008;18:372–7.
3. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer. 1953;6:963–8.
4. Sottoriva A, Barnes CP, Graham TA. Catch my drift? Making sense of genomic intra-tumour heterogeneity. Biochim Biophys Acta Rev Cancer. 2017;1867:95. pii: S0304-419X(17)30001-X.
5. Braakhuis BJ, Leemans CR, Brakenhoff RH. A genetic progressionmodel of oral cancer: current evidence and clinical implications. J Oral Pathol Med. 2004;33:317–22.
6. Cross WC, Graham TA, Wright NA. New paradigms in clonal evolution: punctuated equilibrium in cancer. J Pathol. 2016;240:126–36.
7. Wood HM, Conway C, Daly C, et al. The clonal relationships between pre-cancer and cancer revealed by ultra-deep sequencing. J Pathol. 2015a;237:296–306.
8. Alberts B, Johnson A, Lewis J, et al. Molecular biology of the cell. 4th ed. Garland Science: New York, NY; 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21054/.
9. Ling H, Vincent K, Pichler M, et al. Junk DNA and the long non-coding RNA twist in cancer genetics. Oncogene. 2015;34:5003–11.
10. Pichler M, Calin GA. MicroRNAs in cancer: from developmental genes in worms to their clinical application in patients. Br J Cancer. 2015;113:569–73.
11. Gomes CC, de Sousa SF, Calin GA, Gomez RS. The emerging role of long noncoding RNAs in oral cancer. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123:235–41.
12. Gomes CC, Gomez RS. MicroRNAand oral cancer: future perspectives. Oral Oncol. 2008;44:910–4.
13. Cervigne NK, Reis PP, Machado J, et al. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. Hum Mol Genet. 2009a;18:4818–29.
14. Brito JA, Gomes CC, Guimarães AL, Campos K, Gomez RS. Relationship between microRNA expression levels and histopathological features of dysplasia in oral leukoplakia. J Oral Pathol Med. 2014a;43:211–6.
15. Gibb EA, Enfield KS, Stewart GL, et al. Long non-coding RNAs are expressed in oral mucosa and altered in oral premalignant lesions. Oral Oncol. 2011;47:1055–61.
16. The Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517:576–82. https://doi.org/10.1038/nature14129.
17. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature. 2013;500:415–21.
18. Izumchenko E, Sun K, Jones S, et al. Notch1 mutations are drivers of oral tumorigenesis. Cancer Prev Res. 2015;8:277–86.
19. Stricker T, Kumar V. Neoplasia. In: Kumar V, Abbas AK, Aster JC, editors. Robbins basic pathology. Amsterdam: Elsevier; 2013. 928 p.
20. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL. Cyclin D as a therapeutic target in cancer. Nat Rev Cancer. 2011;11:558–72.
21. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer. 2009;9:153–66.
22. Bunz F. Oncogenes. In: Bunz F, editor. Principles of cancer genetics. Dordrecht: Springer; 2008a. p. 49–74.
23. Bunz F. Tumor suppressor genes. In: Bunz F, editor. Principles of cancer genetics. Dordrecht: Springer; 2008b. p. 77–123.
24. Chow AY. Cell cycle control by oncogenes and tumor suppressors: driving the transformation of normal cells into cancerous cells. Nat Educ. 2010;3:7.

25. Bunz F. Genetic instability and cancer. In: Bunz F, editor. Principles of cancer genetics. Dordrecht: Springer; 2008c. p. 125–70.
26. Giam M, Rancati G. Aneuploidy and chromosomal instability in cancer: a jackpot to chaos. Cell Div. 2015;10:3.
27. Zhang L, Poh CF, Williams M, et al. Loss of heterozygosity (LOH) profiles--validated risk predictors for progression to oral cancer. Cancer Prev Res. 2012;5:1081–9.
28. Hemmer J, Kreidler J. Flow cytometric DNA ploidy analysis of squamous cell carcinoma of the oral cavity. Comparison with clinical staging and histologic grading. Cancer. 1990;66:317–20.
29. Giaretti W, Monteghirfo S, Pentenero M, Gandolfo S, Malacarne D, Castagnola P. Chromosomal instability, DNA index, dysplasia, and subsite in oral premalignancy as intermediate endpoints of risk of cancer. Cancer Epidemiol Biomarkers Prev. 2013;22:1133–41.
30. Sperandio M, Brown AL, Lock C, et al. Predictive value of dysplasia grading and DNA ploidy in malignant transformation of oral potentially malignant disorders. Cancer Prev Res. 2013;6:822–31.
31. Rubio Bueno P, Naval Gias L, García Delgado R, Domingo Cebollada J, Díaz González FJ. Tumor DNA content as a prognostic indicator in squamous cell carcinoma of the oral cavity and tongue base. Head Neck. 1998;20:232–9.
32. Brouns ER, Bloemena E, Belien JA, Broeckaert MA, Aartman IH, van der Waal I. DNA ploidy measurement in oral leukoplakia: different results between flow and image cytometry. Oral Oncol. 2012;48:636–40.
33. Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. Science. 2001;291(5507):1284–9.
34. Yip KW, Reed JC. Bcl-2 family proteins and cancer. Oncogene. 2008;27:6398–406.
35. Fernald K, Kurokawa M. Evading apoptosis in cancer. Trends Cell Biol. 2013;23:620–33.
36. Beatty GL, Gladney WL. Immuneescapemechanisms as a guide for cancerimmunotherapy. Clin Cancer Res. 2015;21:687–92.
37. Economopoulou P, Agelaki S, Perisanidis C, Giotakis EI, Psyrri A. The promise of immunotherapy in head and neck squamous cell carcinoma. Ann Oncol. 2016;27:1675–85.
38. You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell. 2012;22:9–20.
39. Arantes LM, de Carvalho AC, Melendez ME, Carvalho AL, Goloni-Bertollo EM. Methylation as a biomarker for head and neck cancer. Oral Oncol. 2014;50:587–92.
40. Smith IM, Mydlarz WK, Mithani SK, Califano JA. DNA global hypomethylation in squamous cell head and neck cancer associated with smoking, alcohol consumption and stage. Int J Cancer. 2007;121:1724–8.
41. Papillon-Cavanagh S, Lu C, Gayden T, et al. Impaired H3K36 methylation defines a subset of head and neck squamous cell carcinomas. Nat Genet. 2017;49:180–5.
42. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128:683–92.
43. Gomes CC, Fonseca-Silva T, Galvão CF, Friedman E, De Marco L, Gomez RS. Inter- and intra-lesional molecular heterogeneity of oral leukoplakia. Oral Oncol. 2015;51:178–81.
44. Mroz EA, Tward AD, Pickering CR, Myers JN, Ferris RL, Rocco JW. High intratumor genetic heterogeneity is related to worse outcome in patients with head and neck squamous cell carcinoma. Cancer. 2013;119:3034–42.
45. Burrell RA, Swanton C. Tumour heterogeneity and the evolution of polyclonal drug resistance. Mol Oncol. 2014;8:1095–111.
46. Kumar M, Nanavati R, Modi TG, Dobariya C. Oral cancer: etiology and risk factors: a review. J Can Res Ther. 2016;12:458–63.
47. Arduino PG, Bagan J, El-Naggar A, Carrozo M. Urban legends series: oral leukoplakia. Oral Dis. 2013;19(7):642–59.
48. Lodi G, Sardella A, Bez C, Demarosi F, Carrasi A. Interventions for treating oral leukoplakia. Cochrane Database Syst Rev. 2006;4:CD001829.
49. Yalcin E, de la Montes S. Tobacco nitrosamine as culprits in disease: mechanisms reviewed. J Physiol Biochem. 2016;72:107–20.

50. Alexandrov LB, Ju YS, Haase K, Loo PV, et al. Mutational signature associated with tobacco smoking in human cancer. Science. 2016;354:618–22.
51. Grigoryeva ES, Kokova DA, Gratchev AN, Cherdyntsev ES, Buldakov MA, Kzhyshkowska JG, Cherdyntseva NV. Smoking-related DNA adducts as potential diagnostic markers of lung cancer: new perspectives. Exp Oncol. 2015;37(1):5–12.
52. Warnakulasuriya KA, Ralhan R. Clinical, pathological, cellular and molecular lesions caused by oral smokeless tobacco--a review. J Oral Pathol Med. 2007;36(2):63–77.
53. Tilakaratne WM, Ekanayaka RP, Warnakulasuriya S. Oral submucous fibrosis: a historical perspective and a review on etiology and pathogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):178–91.
54. Chattopadhyay A, Ray JG. Molecular pathology of malignant transformation of oral submucous fibrosis. J Environ Pathol Toxicol Oncol. 2016;35(3):193–205.
55. Maserejian NN. Prospective study of alcohol consumption and risk of oral premalignant lesions in men. Cancer Epidemiol Biomarkers Prev. 2006;15:774–81.
56. Setshedi M, Wands JR, de la Monte SM. Acetaldehyde adducts in alcoholic liver disease. Oxid Med Cell Longev. 2010;3:178–85.
57. Zaravinos A. An updated overview of HPV-associated head and neck carcinomas. Oncotarget. 2014;5:3956–68.
58. Ying J, Wang F, Lin J. Human papillomavirus 16 as a risk factor for oral leukoplakia: a meta-analysis. Meta Gene. 2017b;12:43–6.
59. Fakhry C, Psyrri A, Chaturvedi A. HPV and head and neck cancers: sate-of-the-science. Oral Oncol. 2014;50:353–5.
60. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011;29:4294–301.
61. Lingen MW, Xiao W, Schmitt A, Jiang B, Pickard R, Kreinbrink P, Perez-Ordonez B, Jordan RC, Gillison ML. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol. 2013;49:1–8.
62. Bakri MM, Hussaini HM, Holmes AR, Cannon RD, Rich AM. Revisiting the association between candidal infection and carcinoma, particularly oral squamous cell carcinoma. J Oral Microbiol. 2010;2:5780.
63. Gholizadeh P, Eslami H, Yousefi M, Asgharzadeh M, Aghazadeh M, Kafil HS. Role of oral microbiome on oral cancers, a review. Biomed Pharmacother. 2016;84:552–8.
64. Nishisgori C. Current concept of photocarcinogenesis. Phochem Photobiol Sci. 2015;14:1713–21.
65. Park JM, Kang TH. Transcriptional and posttranslational regulation of nucleotide excision repair: the Guardian of the Genome against Ultraviolet Radiation. Int J Mol Sci. 2016;17:1840.
66. Brunotto M, Zarate AM, Bono A, Barra JL, Berra S. Risk genes n head and neck cancer: a systematic review and meta-analysis of last 5 years. Oral Oncol. 2014;50(3):178–88.
67. Siebers TJH, Bergshoeff VE, Otte-Höller I, Kremer B, Speel EJM, van der Laak JAWM, Merkx MAW, Slootweg PJ. Chromosome instability predicts the progression of premalignant oral lesions. Oral Oncol. 2013;49:1121–8.
68. Shridhar K, Walia GK, Aggarwal A, Gulati S, Geetha AV, Prabhakaran D, Dhillon PK, Rajaraman P. DNA methylation markers for oral pre-cancer progression: a critical review. Oral Oncol. 2016b;53:1–9.

Chapter 3
Oral Carcinogenesis and Malignant Transformation

Camile S. Farah, Kate Shearston, Amanda Phoon Nguyen, and Omar Kujan

Introduction

Cancer of the oral cavity and oropharynx is a significant health burden, with over 300,000 new cases diagnosed annually [1]. Oral squamous cell carcinoma (OSCC) constitutes 95% of these malignancies and is mostly preceded by lesions termed oral potentially malignant disorders (OPMDs) that have a high tendency for malignant transformation [2]. Despite advances in diagnosis and treatment modalities, the survival rate of OSCC has not changed significantly in the last five decades [3]. The poor prognosis of oral cancer can largely be attributed to its frequent diagnosis at an advanced stage [4]. Understanding the process and natural history of oral carcinogenesis has the capacity to improve the clinical outcomes of patients with OSCC through early detection and effective OPMD management. This chapter discusses the most recent concepts and knowledge on oral carcinogenesis and malignant transformation of OPMDs.

Oral Mucosa Development and Epithelial Differentiation

Given that OSCC arises from oral epithelium, understanding the normal anatomy, histology, biology and physiology of normal oral epithelial cells is a prerequisite to understanding oral carcinogenesis.

Oral mucosa lines the structures of the oral cavity and developmentally originates from ectoderm and ectomesenchyme—in particular, neural crest cells [5]. Given the sophisticated functionality of the oral mucosa, it has typically been

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subdivided into three categories: lining mucosa, masticatory mucosa and specialised mucosa. Despite this, the mucosa at all oral sites comprises a lining of stratified squamous epithelium (SSE), keratinised or non-keratinised, depending on the region and underlying connective tissue of the lamina propria and submucosa [6, 7].

Lining mucosa covers the lips, cheeks, soft palate, alveolar mucosa, and the floor of the mouth and is typically lined by stratified non-keratinised squamous epithelium. Surface cells are shed continuously and replaced by progenitor stem cells at the basal cell layer. The lamina propria has a similar pattern to that found in the dermis of the skin [6]. On the other hand, masticatory mucosa lines the hard palate and gingiva, while specialised mucosa is unique to the dorsum of the tongue. In both types of mucosa, masticatory and specialised, the lining epithelium is stratified keratinised squamous. They both have elongated rete ridges and relatively dense lamina propria to reinforce the cohesion between epithelium and lamina propria [6, 7].

The oral epithelium has four layers as a result of cell proliferation and sequential differentiation: stratum germinativum (or stratum basale), stratum spinosum (or prickle cell layer), stratum granulosum (or granular layer) and stratum corneum (the keratinised or cornified layer) [6, 7]. The oral epithelium has two cell populations: progenitor (cells are responsible for dividing and providing new cells) and maturing (cells undergo continuing process of differentiation to form a protective barrier) [7]. The final pathway of SSE differentiation allows either reaching entirely cornified dead cells (squames) that are found on the hard palate and gingiva or non-cornified as is seen in lining mucosa. Interestingly, the cytoskeleton of oral epithelial cells has individual intermediate filaments known as cytokeratins (CK) which are products of the CK genes. Cytokeratins are a group of at least 20 subtypes [7]. The phenotype of the cytokeratin reflects the differentiation pathway of the epithelial cells [7]. For example, CK 14 is strongly positive in the basal cell layer. It is important to know that the CK profile changes in the oral epithelium with pathological situations [6, 7].

Embryonic development mostly dictates the distribution of keratinisation in the oral epithelium [8]; however, there are some normal variations seen in adults. In fact, normal proliferation and differentiation of oral epithelium are controlled by autocrine and paracrine factors that are generated by keratinocytes, cytokines, growth factors and circulating systemic factors [6]. Another influencing factor is due to epithelial/mesenchymal interaction [6]. Some researchers argue that mesenchyme plays a significant role in determining the phenotype and morphology of the overlying epithelium. Additionally, gap junctional communication is documented to have a role in regulating oral epithelia differentiation [6]. Moreover, local stimuli in the oral cavity can induce reversible changes depending on the persistence of the stimuli [7]. For example, chronic physical irritation may cause hyperkeratosis of the buccal mucosa. Additionally, inflammation may affect the mitotic activity of oral epithelial cells. Mild subepithelial inflammatory infiltrate may stimulate proliferative activity of the oral progenitor cells, while severe inflammation may cause marked reduction in this activity. These effects probably represent the influence of cytokines [7].

The balance between cell loss and cell formation has a delicate role in stabilising the homeostasis of multilayered oral squamous epithelium [8, 9]. Oral epithelial cells normally divide at different rates, and it is possible to estimate the time necessary to replace all the cells in the epithelium, known as turnover time [8].

Importantly, the oral epithelium divides following a unique pattern forming what is called epidermal proliferation unit. Stem cells divide to transit-amplifying cells that subsequently divide one to five times laterally and upward towards the epithelial surface producing a clone of differentiated cells. Eventually, the epidermal proliferation unit consists of a population of dividing progenitor (transit-amplifying) cells and their respective clones of differentiated cells [6, 9, 10]. It is also evident that proliferation and epithelial turnover increase during wound healing. Surveillance and management systems inside the cell cycle checkpoint will detect damaged cells and will subsequently activate programmed cell death [6, 9]. Exposure to environmental factors can influence the cell cycle phases of oral epithelial cells. Smoking, for example, has the ability to disrupt the cell cycle of normal epithelial cells. Michcik et al. [11] studied the influence of smoking on the cell cycle of normal epithelial cells and showed that the percentage of cells in the individual phases of the cell cycle was significantly dependant on the pack-year smoking history [11].

The oral mucosa displays normal variations that relate to its development and functional demands. Knowledge of development, biology and histology of the epithelial lining of the oral cavity sets a foundation for a better understanding of oral carcinogenesis.

Carcinogenesis

An overwhelmingly large proportion of head and neck cancers are squamous cell carcinomas (SCCs) and variants thereof, originating from the epithelium of the mucosal lining of the upper aerodigestive tract [12]. Two-thirds of these malignancies occur in developing countries; and a high incidence continues to be observed in the Indian subcontinent [13]. Many of these areas are showing rising trends, with a shift to the involvement of younger individuals, and survival rates have improved little or not at all in much of the world over several decades, mainly due to the high risk of developing a second primary cancer [14, 15]. Five-year survival rates are reportedly as low as 9% for some parts of the oral cavity, largely due to late-stage diagnosis when tumour metastasis has occurred [16]. The importance of early detection and prevention of oral cancer and pre-malignancy cannot be overstated. It is imperative to understand oral carcinogenesis and malignant transformation, for the early detection and prevention of oral cancer [4].

Carcinogenesis describes the series of genotypic and phenotypic changes that result in a cell being identified as malignant. Compared with normal cells, cancerous cells display a range of ‘hallmarks’ including resistance to antigrowth signals, evasion of apoptosis, self-sufficiency in growth signals, limitless replicative potential, promotion of angiogenesis, the ability to invade tissue and metastasise, altered

metabolic pathways and the ability to evade the immune system [17, 18]. These cancer hallmarks are functional capabilities that permit cancer cells to survive, proliferate and spread beyond their initial location. Some of these hallmark properties may become activated quite early in the process of carcinogenesis, for example, evasion of apoptosis, while others may only be present in malignant or metastatic tissues. The cancer hallmark concept also describes 'enabling characteristics' which assist in the acquisition and promotion of hallmark capabilities, namely, genomic instability and inflammation [17, 18].

Oral cancer development is a complex, multistep and multifocal process involving field cancerisation and carcinogenesis [14, 19]. In the context of oral cancer, carcinogenesis involves the cells and tissues of the normal oral mucosa transforming into oral squamous cell carcinoma. Oral cancer formation is driven by the accumulation of a series of genetic alterations which activate or inhibit various functions and signalling pathways of the normal oral mucosa, some of which are summarised in Fig. 3.1. Genetic alterations may be driven by risk factors such as tobacco smoking or alcohol consumption or by genetic susceptibility. The concept of field cancerisation, as conceived by Slaughter et al. in 1953 [20], also known as *field defect* or *field effect*, describes the process by which a large area of tissue becomes genetically but not histologically altered and is more susceptible to malignant transformation [20]. Oral cancer, like carcinomas in other tissues, develops over many years, and during this period, there may be multiple sites of neoplastic transformation

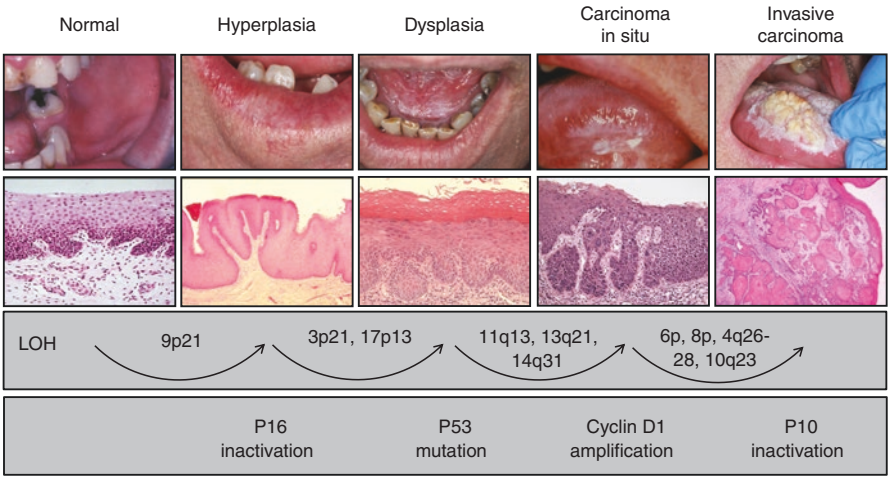

Fig. 3.1 Clinical, histological and molecular models of oral carcinogenesis. Oral carcinogenesis can be understood clinically, histologically and molecularly. The top panel depicts the physical manifestations of premalignant and cancerous lesions in comparison to normal mucosa. The images below display the histological changes that occur to normal oral mucosa as it progresses through hyperplasia, dysplasia, carcinoma in situ and invasive carcinoma. The grey boxes below depict the accumulation of mutations that occur during carcinogenesis, with characteristic regions undergoing loss of heterozygosity (LOH) (above) and critical mutations correlated with premalignant and malignant lesion stage (below). Image used with permission from C. S. Farah et al. (eds.), Contemporary Oral Medicine, Springer Nature Switzerland AG 2019

occurring throughout the oral cavity [14]. Thus, multicell anaplastic tendency results in a multifocal development process of cancer at various rates within the entire field, with subsequent development of abnormal tissues around a tumourigenic area, the areas of which may later coalesce and create atypical areas, even after complete surgical removal [21]. This may partly explain the presence of second primary tumours and recurrences [21]. The continual presence of mutations may also signify changes in DNA repair and apoptosis, thereby increasing the susceptibility to future transformation [22]. Mutational adaptations that modify the survivability of particular clones of transforming cells may also further enhance the level of resistance to therapeutic control [14, 22]. A recent genetic analysis revealed that cancers developing at distant sites within the oral cavity often are derived from the same initial clone [23]. The multiplicity of the oral carcinogenesis process makes it difficult to interrupt the progression to cancer through the surgical removal of a premalignant lesion alone [14]. It is important to recall that multifocal presentation and mutational expression of tumour suppressor genes may also be the consequence of long-term exposure to various environmental and exogenous factors [14].

Clinical Model of Carcinogenesis

Clinically, human tumours can be broadly divided into three groups: premalignant lesions, primary tumours and metastasis, the former of which is our focus. An oral premalignant lesion is an area of morphologically or genetically altered tissue that is more likely than normal tissue to develop cancer. Estimates of the global prevalence of OPMD range from 1% to 5% [24]. Cells in premalignant lesions are clonally expanded because of the acquisition of selective growth advantage by genetic alteration (or alterations) that occur in cells, and these initiated cells may be less responsive to negative growth regulators and cell differentiation inducers [21]. The initiated cells or normal cells convert to malignant cells (converted cells) by additional or multiple genetic alterations and produce primary tumours [21]. A range of tissue and cellular alterations consistent with carcinoma commonly precedes OSCC, but when it remains restricted to the surface epithelial layer, it is a potentially malignant condition termed oral epithelial dysplasia (OED) [16, 25]. Although OSCC is not linear in its development, there is general agreement that it begins as a simple epithelial hyperplasia and progresses through OED, with more severe dysplastic changes signifying more extensive genetic aberrations [16]. The timeframe for this process is not known but is thought to be a relatively slow process, with malignant transformation occurring within 10 years [16]. The reported rates of malignant transformation of leukoplakia range from <1% to 18%, and various factors, such as the location within the oral cavity, clinical appearance (homogenous versus non-homogenous) and the presence of dysplasia, correlate with the risk of progression [26].

There are clinically apparent premalignant lesions of oral cancer. They include leukoplakia, erythroplakia, oral lichen planus and oral submucous fibrosis. The

most recent definition of leukoplakia emerged from a World Health Organization-supported workshop as 'a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer' [25]. This definition should exclude such white lesions as traumatic or smokers' keratosis, which do not carry an excessive risk of OSCC [16]. Leukoplakia is seen most frequently in middle-aged and older males, with an increasing prevalence with age. Fewer than 1% of males below the age of 30 have leukoplakia, but the prevalence increases to 8% in men over the age of 70, while the prevalence in females past the age of 70 is approximately 2% [14].

Similar to the definition for leukoplakia, erythroplakia is a clinical term that refers to a red patch that cannot be defined clinically or pathologically as any other condition. This definition should exclude inflammatory conditions that may result in a red clinical appearance. Oral erythroplakia occurs most frequently in older males and appears as a red macule or plaque with a soft, velvety texture, with the floor of mouth, lateral tongue, retromolar pad and soft palate being the most common sites of involvement [25]. Some lesions may be intermixed with white, and this is termed an erythroleukoplakia.

The most common site for intraoral carcinoma is the tongue, which accounts for around 40% of all cases in the oral cavity proper [13]. The floor of the mouth is the second most common intraoral location, with less common sites including the gingiva, buccal mucosa, labial mucosa and hard palate (Warnakulasuriya [13]). A two-tier system has been developed by Kujan et al. [27] that categorises OED into low and high risk of undergoing malignant transformation, in an attempt to make histopathology more practical for the clinician [27]. Leukoplakic lesions in high-risk sites should be considered to be at high risk of malignant transformation.

Histopathological Model of Carcinogenesis

Although diagnosis of invasive OSCC is largely uncomplicated, pathologic diagnosis of oral premalignant lesions can be perplexing. The histological finding of dysplasia is strongly associated with an increased rate of invasive cancer development. The World Health Organization (WHO) has established criteria for dysplasia, including the architectural and cytological changes in the epithelium which are summarised in Table 3.1 [28]. Visual representations of histological examples of carcinogenesis are summarised in Fig. 3.1.

The classic WHO oral dysplasia grading system includes diagnoses of no dysplasia, mild dysplasia, moderate dysplasia and severe dysplasia. In cases of mild dysplasia, cytological and architectural changes are confined to the lower third of the thickness of the epithelium; in cases of moderate dysplasia, changes are seen in up to two-thirds of the thickness of the epithelium. In cases of severe dysplasia, the dysplastic changes fill more than two-thirds of the thickness but less than the entire thickness of the epithelium. The dysplastic cells of carcinoma in situ occupy the entire thickness of the epithelium (bottom to top changes), although the basement

Table 3.1 Characteristic architectural and cytological changes for oral epithelial dysplasia grading [28]

Architectural changes	Cytological changes
Irregular epithelial stratification	Abnormal variation in nuclear size (anisonucleosis)
Loss of polarity of basal cells	Abnormal variation in nuclear shape (nuclear pleomorphism)
Drop-shaped rete ridges	Abnormal variation in cell size (anisocytosis)
Increased number of mitotic figures	Abnormal variation in cell shape (cellular pleomorphism)
Abnormal mitoses not limited to basal or parabasal layers	Increased nuclear-cytoplasmic ratio
Premature keratinisation in single cells (dyskeratosis)	Increased nuclear size
Keratin pearls within rete ridges	Atypical mitotic figures
	Increased number and size of nucleoli
	hyperchromasia

membrane is still intact. Invasive SCC involves dysplastic cells invading the underlying connective tissue stroma through the basement membrane.

It should be emphasised that the assessment of dysplasia is subjective and open to interpretation, and accurate pathologic grading of dysplasia requires ample experience [29, 30]. Additionally, the merit of OED grading in predicting malignant transformation is limited by the virtue that not all OED lesions transform into malignancy [30]. On the contrary, some OED lesions may regress [24].

Animal Model of Oral Carcinogenesis

Animal models have provided a useful tool to investigate the process of carcinogenesis, the development and growth of tumours and the influence of carcinogens/risk factors and potential treatments. The use of knockout or transgenic mice is a powerful tool to understand the role of specific genes in tumour growth and development [31–33]. The most widely used animal models for oral carcinogenesis are the hamster cheek pouch model and the 4-nitroquinoline 1-oxide-(4-NQO)-induced oral (tongue) carcinogenesis model [32]. These models attempt to mimic the histological, molecular and immunological characteristics of human oral carcinogenesis as closely as possible.

Induction of SCC in the cheek pouch of hamsters was first described with the aid of three polycyclic aromatic hydrocarbons, namely, 7,12-dimethylbenz(a)anthracene (DMBA), 20-methylcholanthrene (20-MC) and 3,4-benzpyrene [14]. DMBA is a widely used carcinogen in experimental oral carcinogenesis. The DMBA model was first utilised by Salley in 1954 and involves application of DMBA (0.5%), to the hamster cheek pouch for a period of 16 weeks, which results in the formation of

invasive OSCC. The model is analogous to what is observed in humans as it shows a gradual development of precancerous lesions, which become dysplastic and undergo malignant transformation to invasive oral squamous cell carcinoma [34]. A range of variants have been developed utilising the basic model combined with other carcinogens such as alcohol, tobacco or alternate chemicals or knockout or transgenic animals.

DMBA-treated hamsters demonstrate expression of oncogenes also seen in human OSCC such as c-*erb*-B (gene for EGRF protein), c-Ha-*ras* (gene for p21 protein), Ki-*ras* and mutant p53 [35]. Expression of c-Ki-ras is of particular interest as it appears at an early stage of tumour development, but not in the healthy oral mucosa [14]. These animals display metabolic markers comparable to human lesions, namely, γ-glutamyltranspeptidase, ornithine decarboxylase and polyamines [36, 37]. A recent study performed a comparative immunohistochemical study using the hamster model and human normal and OSCC tissues and found that DMBA-induced changes in protein expression in the hamster were largely mirrored in human cancer samples. This took the form of activation of cytochrome P450, increased markers of oxidation and changes in the expression of a range of proteins involved in apoptosis, angiogenesis, proliferation and invasion [38, 39]. This model was also used to identify a novel tumour suppressor gene (TSG) named DOC-1 via a negative screen. Healthy hamster oral keratinocytes express DOC-1, while keratinocytes from DMBA-treated hamsters display mutations that lead to very low levels of DOC-1 protein [40]. Re-expression of DOC-1 in malignant oral keratinocytes results in reversion of many malignant phenotypes to normal, thus causing the DOC-1 transfected oral cancer cells to look and act like their normal counterpart [41]. The precise function of DOC-1 in normal oral keratinocyte biology is still being investigated, but it appears to act as a cell cycle regulator and be involved in apoptosis and its expression is lost in approximately 70% of human SCC [38, 42]. Other animal models for the study of oral carcinogenesis include those in rats and mice using water-soluble 4-NQO, which produce tongue lesions including squamous cell neoplasms within 32 weeks and palatal tumours within 49 weeks [14, 32]. Typically, this model includes an induction period of between 8 and 32 weeks, where 4-NQO is supplied in the drinking water, and then a development period of 15–24 weeks, where animals are fed untreated water and tongue lesions are allowed to develop. In the mouse, 10 weeks of DMBA treatment results in the majority of animals displaying lesions greater than grade 2 by week 25, with 75% of these histologically assessed as severe dysplasia, CIS or an invasive SCC [43]. In the rat, an 8-week induction generates tongue lesions including leukoplakia and papillary tumours by 32 weeks. Histologically, these lesions are predominantly hyperplastic, with some displaying mild and moderate dysplasia, but with 32 weeks of 4-NQO treatment (and 24 weeks development), the lesions display frequent severe dysplasia (80%), a 15% incidence of CIS and a rate of invasive SCC ranging from 50% to 70% [44]. Increased levels of polyamine synthesis, as well as nucleolar organiser regions (NORs), have been noted in this rat model with the progression of oral carcinogenesis [14]. Rats carrying human c-Ha-Ras proto-oncogene develop 4-NQO-induced cancer more rapidly than wild type, suggesting c-H-Ras alterations are involved in the mechanism of 4-NQO [45].

Lesions induced by 4-NQO display molecular similarities to human oral lesions. A recent RNA-seq study found increased transcript associated with matrix breakdown and migration, proliferation and cell cycle, hypoxia signalling and DNA replication compared with untreated controls [43]. This was reflected at a protein level with increased protein expression via immunohistochemistry in MMP-9, beta-catenin and markers of oxidative stress [43]. In another study by Foy et al., utilised RNA-seq to compare gene expression profiles between normal, hyperplastic, dysplastic and cancerous mouse tongue tissue generated using the 4-NQO model and correlated these with human cancer datasets. They were able to identify 'early' gene sets correlated with hyperplasia, 'intermediate' gene sets correlated with dysplasia and 'late' gene sets correlated with malignant transformation as well as a 'progressive' gene set where expression increased over the course of experimental carcinogenesis [46]. Overall, there were changes in signalling via NFKB pathway (early), the MAPK/ERK pathway (early and late), IL4 signalling and several apoptosis pathways. The 4-NQO model is characterised by frequent CASP8 mutations and amplifications at 11q13.3 and is potentially sensitive to MEK inhibition [46]. It is similar to a subset of human tumours characterised in TCGA that are HPV negative and display inactivating CASP8, activating HRAS and wild-type TP53 [47].

Molecular Model of Carcinogenesis

Genetic alterations define the molecular basis of carcinogenesis, and these include point mutations, amplifications, rearrangements, copy number variations, insertions and deletions as well as chromosomal translocations of entire exomes or genomes.

Oncogenes, gain-of-function mutations of highly regulated normal cellular counterparts (proto-oncogenes), are likely involved in the initiation and progression of oral neoplasia. Genetic damage in oral cancer cells can be divided into two categories: dominant changes most frequently occurring in proto-oncogenes but also in certain tumour suppressor genes (TSGs) result in gain of function and recessive changes, mutations most frequently noted in growth inhibitory pathway genes or commonly in TSGs, cause loss of function [21]. Cellular oncogenes were initially discovered by the ability of tumour cell deoxyribonucleic acid (DNA) to induce transformation in gene transfer assays, and these experiments have led to the identification of more than 60 cellular oncogenes [48]. Mechanism of activation of these cellular oncogenes includes point mutations and DNA rearrangements. As summarised in Fig. 3.1, several oncogenes have been implicated in oral carcinogenesis.

For human oral cancer, more than 63 karyotypes have been described and commonly reported to be associated with oral carcinogenesis among them are recurrent chromosome 9, 13, 18 and Y deletions and cytogenetic alterations in cellular oncogenes B-cell lymphoma-1, int-2 and hst which have been mapped to chromosome Iq [49]. Approximately two-thirds of all head and neck cancer cells contain a deleted region located in chromosome 9p21-22, which appears in dys-

plastic and carcinoma in situ lesions, thereby suggesting that gene in this region is knocked out early in carcinogenesis [50]. Frequently deleted also are chromosomal regions in 3p and 13q [19]. Aberrant expression of the proto-oncogene epidermal growth factor receptor (EGFR, c-erb 1), members of the ras family, as well as c-myc, int-2, hst-1, PRAD-1 and bel, is believed to contribute to oral carcinogenesis [50]. Glutathione *S*-transferase M1 (GSTM1)-null genotype appears to be the most consistent polymorphic susceptibility marker for head and neck cancer (Aida et al. [140]). *ALDH1B* and *ALDH2* (aldehyde dehydrogenase 2) genes are also associated with HNSCC and show significant correlation with alcohol consumption [51].

Califano et al. [19] demonstrated the most common allelic events in a large number of primary preinvasive lesions and invasive HNSCC to develop a molecular progression model, which involves the inactivation of many putative suppressor gene loci. Chromosomes 9p and 3p appear to be lost early, closely followed by loss of 17p [52]. Mutations in p53 gene are seen in the progression of preinvasive to invasive lesions. Other genetic events, such as amplification of cyclin D1 and inactivation of p16, have been tested predominantly in invasive lesions, but their precise order in the model was not determined.

More than 50% of all primary HNSCC harbour a p53 mutation, and inactivation of p53 represents the most common genetic change in all human cancers. The importance of p53 in a larger percentage of cancers may be shown following identification of members of its suppressor pathway, which themselves may be altered. In normal cell biology, p53 acts as a regulator of DNA synthesis, and when genomic DNA is damaged, p53 is produced to block cell division at the G1-S boundary and stimulate DNA repair and activate pathways leading to apoptosis [19]. Mutation of p53 allows a tumour to pass through G1-S boundary and propagate genetic alterations that lead to other activated oncogenes or inactivated TSGs [19]. The most commonly deleted region in head and neck cancer is located at chromosome 9p21–22 [53]. Loss of chromosome 9p21 occurs in the majority of invasive tumours in head and neck cancer, and frequent homozygous deletions in this region represent one of the most common genetic changes identified [53]. p16 (*CDKN2*) present in this deleted region is a potent inhibitor of cyclin D1, and this loss of p16 protein has also been found in most advanced premalignant lesions and is important in early malignant progression [54]. The loss of chromosome 17p is also frequent in most human cancer and is seen in approximately 60% of invasive lesions [52]. Although p53 inactivation correlates closely with loss of 17p in invasive lesions, p53 mutations are quite rare in early lesions that contain 17p loss [52]. Loss of chromosome arm 10 and 13q is also noted in primary tumours [19].

Loss of function of the tumour suppressor p53 can result in uncontrolled cell division and progressive genomic instability, such as the loss of heterozygosity (LOH) and microsatellite instability (MSI).

Chromosome 9p21 containing p16 tumour suppressor gene is frequently lost in HNSCC and oral preneoplastic lesions. Chromosome 3p14 contains the tumour

suppressor gene fragile histidine triad (FHIT) as well as a common fragile site, FRA3B which is also found to be frequently deleted in early tumourigenesis, and its deletion is associated with exposure to cigarette smoke [55].

Stimulating oral keratinocyte proliferation are growth factors, and during oral carcinogenesis, growth factors are deregulated through increased production and autocrine stimulation [49]. Transforming growth factor-alpha (TGF-alpha) is overexpressed early in oral carcinogenesis by hyperplastic epithelium and later by the inflammatory infiltrate, particularly eosinophils, surrounding the oral epithelium, and in head and neck cancer patients who later develop a second primary cancer, normal oral mucosa oversecretes TGF-alpha, suggesting a premalignant state of rapid proliferation and genetic instability of the epithelium [49]. Concomitant expression of TGF-alpha and EGFR may indicate more aggressive tumours than those overexpressing EGFR alone [49].

EGFR is the biological receptor of EGF and TGF-alpha, and malignant oral keratinocytes possess between 5 and 50 times more EGFR than their normal counterparts [56]. Oral tumours overexpressing EGFR exhibit a higher proportion of complete responses to chemotherapy than tumours with low-level EGFR expression. Overexpression of EGFR, presumably due to higher intrinsic proliferative activity, could result in higher sensitivity to drug therapy cytotoxic to cells undergoing mitogenesis [56]. Though not fully understood, several mechanisms have been postulated to activate EGF genes in carcinogenesis [49] including deletions or mutations in the N-terminal ligand-binding domain such as those occurring in the viral oncogene verb B, overexpression of the EGFR gene concurrent with the continuous presence of EGF and/or TGF-alpha or deletion in the C-terminus of the receptor, which prevents downregulation of the receptor after ligand binding.

Also important in inhibiting oral keratinocyte proliferation are cell surface molecules such as E-cadherin, a cell-to-cell adhesion molecule associated with both division and metastasis, which is downregulated in oral cancers, and DOC-1, an N-Cam-like molecule believed to be an important cell-to-cell contact inhibitor that is mutated during oral cancer development [40].

Transcriptional factors, or proteins that regulate the expression of other genes, are also altered in oral carcinogenesis, and this alteration of intracellular pathways modulates gene expression. The transcription factor c-myc, which helps to regulate cell proliferation and differentiation, is frequently overexpressed in oral cancer, and this is most frequently associated with poorly differentiated tumours and with poor prognosis (Shpitzer et al. [50]). Genes whose expression is stimulated by c-myc and their significance to oral carcinogenesis are being studied [49]. Also amplified in oral cancer is another important transcription factor, the cell cycle promoter PRADI (also CCNDI or cyclin Dl), whose importance to oral carcinogenesis is being investigated [48].

Growth suppressor intracellular messengers may include the adenomatous polyposis coli (*APC*) gene, a G-like protein frequently mutated in certain familial colorectal cancers, and the *APC* gene may be altered in premalignant oral lesions [50].

Factors Influencing Oral Carcinogenesis

Risk Factors

The incidence of oral cancer has significant local variation. The geographical patterns of oral cancers have been presumed to be due to the varying prevalence of risk factors among countries, in particular tobacco and alcohol consumption and the diet quality [57]. Similarly, the variations by ethnicity are largely due to the social and cultural practices and the influence of dietary and genetic factors [57]. Numerous risk factors or possible causative agents have been described, which are covered in detail in other chapters. It is important to note that the presence of one risk factor enhances the effects of a second risk factor and development of OPMD and oral cavity cancer. This concept is termed synergism. In a study by Kadashetti et al. [58], an odds of 2.2 times more of developing OPMD cases was observed for a combination of risk factors, that is, smoking, tobacco quid chewing and alcohol drinking, as compared to non-chewers, non-smokers and non-drinkers [58].

While it is traditionally assumed that OPMD risk factors are similar to OSCC risk factors, a proportion of OPMD and OSCC cases occur in the complete absence of any identifiable risk factor, particularly in young never smokers affected by these diseases [59]. Our understanding of OPMD aetiology is incomplete, and there is an urgent need for further research into predisposing factors.

A cross-sectional study by Kumar et al. [60] in 2015 found that 13.7% of the Indian population studied showed the presence of OPMD, and of these, oral submucous fibrosis was the most prevalent and erythroplakia the least prevalent. In this study, males were found to have a significantly higher prevalence of OPMD compared to females, presumably due to an increased number of males with smoking and smokeless tobacco usage. No significant differences were found on socioeconomic status, toothbrushing methods or brushing frequency [60].

Tobacco and Smokeless Tobacco

The various forms of tobacco use can vary across geographical areas and cultures around the world, and in the United States, Europe and Australia, cigarettes, cigars and pipes are the major types of smoking tobacco, while chewing tobacco and areca nut, also known as betel nut, are the most common forms of smokeless tobacco, predominantly used in India, Pakistan, China and other areas of Asia. Smokeless tobacco is used either alone or as part of a concoction and in a myriad of forms including betel quid, bidis, paan, naswar or nass [61]. Although the use of electronic nicotine delivery systems (or ‘ENDS’), also known as e-shisha, e-cigars, e-pipes, e-Hookas, hookah-pens, vape-pipes and e-cigs are commonly advocated as a means of tobacco harm reduction [62], although there is building evidence that they are not safe and may still contribute to oral carcinogenesis causing DNA strand breaks and cell death [63, 64].

Smoking and smokeless tobacco use are well-accepted risk factors for developing oral cancer and are implicated in a large majority of squamous cell carcinomas in the head and neck region. The risk for squamous cell cancer of the head and neck is estimated to be approximately tenfold greater for current smokers compared with never smokers [65]. This risk decreases with time from cessation of exposure, although the risk never reduces to the level of a never smoker [65]. For persons with an OPMD relative to individuals with a benign oral tissue condition, Li reported that the adjusted OR for current smoking was 4.32 (95% CI, 1.99–9.38), while for former smokers, the OR was 1.47 (95% CI, 0.67–3.21), each OR relative to never smokers [66]. A reduction in tumour suppressor activity by the gene and the development of mutations in p53 are associated with smoking and an increased risk for oral carcinoma development [14, 49]. Across anatomic tumour sites, the NFE2L2 oxidative stress pathway is a tobacco-related signature. Areca nut, also known as betel nut, use has been associated with the development of OPMD, and various studies from around the world have reported the adverse effects of areca nut chewing. Some areca nut-specific nitrosamines suspected to be carcinogenic are 3-methylnitrosamino propionaldehyde (MNPA), 3-methylnitrosamino propionitrile (MNPN), *N*-nitrosoguvacine (NGC) and *N*-nitrosoguvacoline (NGL). MNPA in particular causes DNA single-strand breaks and DNA protein cross links [60].

The literature reports an odds ratio of between 8.4 and 41 for developing OPMD in tobacco/areca nut chewers as compared to non-chewers [58]. The highest odds ratio is found in India, and it is plausible that this difference could be due to the differences in habits practiced by different study populations and also the composition and method of chewing which varies from country to country [58]. As the duration of tobacco/areca nut chewing increases, the risk of developing OPMD and oral cancer increases in a dose-dependent manner [58]. A recent systematic review of 18 case-control studies reported that betel quid with tobacco chewing carried the highest risk for developing OPMD compared with other forms of smokeless tobacco use [67].

Alcohol

Numerous studies have suggested that alcohol is a risk factor for oral cancer. Individuals consuming more than 170 g of whisky daily have ten times higher risk of oral cancer than light drinkers [68]. Alcohol may have an additive effect, and it has been suggested that it facilitates the entry of carcinogens into exposed cells, altering the metabolism of oral mucosal cells [68]. Therefore, alcohol consumption and tobacco use can have a synergistic effect on cancer risk. It has been suggested that the incidence of p53 mutation is much higher in patients who are exposed to both tobacco and alcohol versus non-users. A study in 2008 implicated the ADH1C*2/*2/MTHFR 677TT genotype combination as more susceptible for developing OSCC, with a 20-fold increase in risk in heavy drinkers and a 5.9- and 2.8-fold increase in risk, respectively, in moderate drinkers and light drinkers [69].

Comparatively, few epidemiologic studies, however, have investigated the role of alcohol in relation to OPMD risk [66]. Findings from investigations into the association between alcohol consumption and either oral leukoplakia or other potentially premalignant oral diagnoses have not been consistent across the studies [70]. There is uncertain consensus that overall alcohol consumption is associated with increased OPMD risk. Some studies of premalignant lesions and OED have found that risks associated with drinking are dependent upon the level of alcohol intake, with the highest risks observed for the highest level of alcohol intake; however, after adjusting for various potential confounders, one study found no evidence of an increased OMPD risk even among those persons who consumed >20 drinks/week [66]. Previous studies have reported that the risk of oral cancer and OPMD can vary by beverage type (beer, wine and hard liquor); however, that same study found little evidence that any type of alcoholic beverage consumption was associated with an increased OPMD risk [66, 70]. In contrast, a risk factor model reported that betel-quid chewing and consumption of alcohol were the only statistically significant characteristics for OPMD risk after controlling for other factors [71]. After controlling for all other variables, the adjusted OR for daily chewers was 10.1 (95% CI, 3.4–29.7), with a strong dose-response relation, and when considering the consumption of alcohol and risk of OPMD, the adjusted OR for weekly drinkers was 2.7 (95% CI, 1.2–6.3) [71].

The risk of oral cancer formation in chronic users of alcohol containing mouth rinses is controversial [72], but there appears to be building evidence that the use of high alcohol-containing mouthwashes has a synergistic effect with tobacco smoking and is more likely to add to the risk profile of patients displaying OED [73], with a greater proportion of mouth rinse users displaying dysplasia on histopathology, although this did not reach statistical significance [74].

Viruses

Much research is being performed to determine the role of oncogenic viruses in human cancer, and it is an emerging area of study. Viruses are capable of hijacking host cellular apparatus and modifying DNA and chromosomal structures and inducing proliferative changes in cells [68]. In particular, the human papilloma virus (HPV) and the herpes simplex virus (HSV) have been implicated in forms of oral cancer.

The role of some HPV subtypes in the aetiopathogenesis of OPMD is controversial; however, an epidemiological association between HPV and OMPD has been reported [75]. A systematic review found that HPV detection rate is higher in the OPMD group than in the controls (OR = 3.87; 95% CI, 2.87–5.21), suggesting a potentially important causal association between HPV and OPMD [75]. The most commonly detected HPV in the head and neck squamous cell carcinoma (HNSCC), detected in 90–95% of HPV-positive cases, is HPV-16, followed distantly by HPV-18, HPV-31 and HPV-33 [68]. The HPV type most commonly detected in OPMDs has been reported to be HPV-16, 18 with HPV-6, 11 found in only a few studies

[75]. In a subgroup analysis of OPMD, Syarjanen et al. calculated the pooled estimates of the odds ratio (OR) for the association of HPV with OPMD, when compared with healthy oral mucosa, and reported that HPV was associated with oral leukoplakia (OR = 4.03; 95% CI, 2.34–6.92), oral lichen planus (OR = 5.12; 95% CI, 2.40–10.93) and epithelial dysplasia (OR = 5.10; 95% CI, 2.03–12.80) [75]. HPV-positive oral and oropharyngeal cancer are a distinct clinico-pathological entity and are less likely to occur among heavy smokers and drinkers and have lesser likelihood of p53 mutation. While the prognostic significance of HPV in precancerous oral lesions is not clear, most studies have reported improved disease-specific survival and a better prognosis for HPV-positive oropharyngeal cancer [68]. It has been suggested that HPV-positive tumours may have better prognosis by inactivating retinoblastoma (Rb) [68].

Similarly, the role of HSV in the aetiopathogenesis of OPMD is controversial; however, there may be an epidemiological association between HSV and OMPD [76]. Epidemiological studies have showed higher levels of IgG and IgM antibodies in oral cancer patients as compared to control subjects. A population-based study reported that HSV1 antibody positivity was associated with a slightly increased risk of OSCC (adjusted odds ratio (OR), 1.3; 95% confidence interval (CI), 0.9–2.0) and concluded that the presence of HSV1 may increase the risk of OSCC in individuals who were already at increased risk due to cigarette smoking or HPV infection [77].

Risk of oral cavity and pharyngeal cancer is twofold higher among human immunodeficiency virus (HIV) patients indicating an association between HIV and OSCC [68]. Epstein-Barr virus (EBV), human herpesvirus-8 (HHV-8) and cytomegalovirus have also been reported as risk factors of OSCC in different studies [68]. A recent study assessing the immunohistochemical expression of Epstein-Barr virus latent membrane protein 1 among OPMD, OSCC and healthy controls found that there was no significant association between Epstein-Barr virus positivity and OPMD and OSCCs [78].

Candida

Candida species have been suggested to play a role in oral carcinogenesis; in particular, *C. albicans* have been implicated in the development of OPMDs. However, the pathogenesis is not well understood and is still a field under research.

The ability of *C. albicans* to colonise, penetrate and damage host tissues depends upon the imbalance between *C. albicans* virulence factors and host defences, often due to specific defects in the immune system [79]. Cell surface proteins called adhesins mediate adherence of *C. albicans* to other microorganisms and host cells. The contact to host cells triggers phenotypic switching from the yeast form to hyphae form, which directs production of carcinogenic compounds, like the nitrosamine *N*-nitrosobenzylmethylamine [80]. Strains with high nitrosation potential have been isolated from lesions with more advanced precancerous changes, and the yeast cells in such cases extend from the mucosal surface to the deeper epithelial cell layers, representing transport and deposition of precursors (like nitrosamines)

to deeper layers [80]. Carcinogenic compounds can then bind with DNA to form adducts causing miscoding or irregularities in DNA replication. Certain strains of *C. albicans* may play a key role in the development of dysplasia [80].

It has also been shown that the epithelium of the chick embryo, when infected with *C. albicans*, shows squamous metaplasia and a higher proliferative phenotype [68]. Leukoplakia with candidal infection has been reported to have a higher rate of malignant transformation than uninfected leukoplakia [81]. The causal association of *Candida* species and oral cancer is controversial and requires further study.

Inflammation

Cytokines seen in inflammation, including interleukins (ILs), tumour necrosis factors (TNFs) and certain growth factors, are an important group of proteins that regulate and mediate inflammation and angiogenesis, and when there is a downregulation in their production, tumour growth, invasion and metastasis are facilitated [14]. A putative correlation has been raised by some genetic association studies between functional DNA polymorphisms in cytokine genes and oral cancer [14]. When compared to controls, patients with oral cancer demonstrate increased serum levels of proinflammatory cytokines, interleukin (IL)-1β, IL-6, IL-8 and TNF-α, as well as the anti-inflammatory cytokine, IL-10 [14]. The anti-inflammatory cytokine IL-4 inhibits oral cancer invasion by the downregulation of matrix metalloproteinase-9 [14].

Genetic Predisposition

Genetic predisposition and family history have been shown to play a role in head and neck cancer, related to polymorphisms in carcinogen-metabolising enzyme systems. A recent extensive meta-analysis pooled individual-level data across 12 case-control studies including 8967 HNC cases and 13,627 controls; after adjusting for potential confounding factors, a family history of head and neck cancer in first-degree relatives increased the risk (OR = 1.7, 95% CI 1.2–2.3) [57]. The risk was higher when the affected relative was a sibling (OR = 2.2, 95% CI 1.6–3.1) rather than a parent (OR = 1.5, 95% CI 1.1–1.8) and for more distal head and neck sites (hypopharynx and larynx) [57]. The OR rose to 7.2 (95% CI 5.5–9.5) among subjects with a family history, who were alcohol and tobacco users, and no association was observed for a family history of nontobacco-related neoplasms and the risk of HNC (OR = 1.0, 95% CI 0.9–1.1) [57]. Other estimates of risk in first-degree relatives of head and neck cancer patients range from 1.1 to 3.8 [59].

Polymorphic variation of genes in the xenobiotic metabolism pathways such as in *CYP1A1* or the genes coding for glutathione *S*-transferase-M1 and N-acetyltransferase-2 may be implicated [59]. Individuals that carry the fast-metabolising alcohol dehydrogenase type 3 (*ADH3*) allele may be particularly vulnerable to the effects of chronic alcohol consumption and could be at increased risk

to develop oral cancer [59]. The single-nucleotide polymorphism A/G870 in the *CCND1* gene that encodes Cyclin D is associated with susceptibility to oral cancer. The AA genotype or the GG wild-type genotype may increase risk for oral cancer [59].

A recent review reported increased susceptibility for OPMD risk with single-nucleotide polymorphisms (SNPs) in *GSTM1 (null)*, *CCND1 (G870A)*, *XPD (codon 751)* and *MMP3 (-1171; promotor region)*, common in majority of populations (Asians, Caucasians, Brazilians and others) [82]. However, the risk associated with SNP in *p53 (codon 72)* was restricted to Indian populations, and it was hypothesised that the high prevalence of SNP in *p53 (codon 72)* may be partly responsible for higher incidence of OPMD in this population [82]. It is possible this may be a chance association, with p53 being the most commonly inactivated tumour suppressor gene in the development of oral cancer. *Gemin3 (rs197412 C/T)* on the other hand was found to be associated with reduced risk for OPMD in Indian and Caucasian populations [82].

Genetic Instability Syndromes

A cancer syndrome is a genetic disorder in which inherited generic mutations predispose affected individuals to developing multiple independent primary tumours, and these individuals carry a high lifetime risk of developing cancer. Tumour suppressor genes are involved in controlling cell growth, both by acting as gatekeepers and inhibiting cell proliferation and promoting cell death, and by acting as caretakers, maintaining the integrity of the genome by DNA repair mechanisms [83]. Mutations of these genes are implicated in the development of cancer syndromes, and other genes that may be affected include DNA repair genes, oncogenes and genes involved in angiogenesis.

There are several genetic diseases that have a genetic instability phenotype and a higher frequency of carcinogenesis. In disorders such as xeroderma pigmentosum, ataxia-telangiectasia, Bloom's syndrome and Fanconi anaemia, where there are defective caretaker genes, there is an increased incidence of second primary malignancies, including oral cancer (Prime et al. [83]). By contrast, with the exception of Li-Fraumeni syndrome, abnormalities of gatekeeper genes do not usually predispose to oral cancer [83]. The protein produced by the *TP53* gene, p53, is involved in cell cycle arrest, DNA repair and apoptosis [84]. Defective p53 may not be able to properly perform these processes, which may be the reason for tumour formation in Li-Fraumeni patients. Because only 60–80% of individuals with the disorder have detectable recessive mutations in *TP53*, other mutations in the p53 pathway may be involved. These include MDM2 overexpression and *CDKN2A* deletion [83].

About 1% of the general population is heterozygous for *ATM* mutations such as that noted in Ataxia telangiectasia (AT) patients [84]. *ATM* is important in activating p53 in response to DNA damage. Variants of AT are caused by mutations in *NBS* and in MRE11A [84].

Bloom's and Werner's syndromes have a defect in genomic stability in common. The genes mutated in these syndromes, *BLM* and *WRN*, respectively, are highly homologous to RecQ helicase. The predominant form of mutations is gross DNA deletions [84]. Both *BLM* and *WRN* are associated with processing the structures associated with stalled replication forks [84].

The cells from patients with Fanconi anaemia (FA) display high levels of chromosomal instability and are hypersensitive to mitosis-inducing cross-linking agents [84]. The genes known to cause FA are FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ, FANCL, FANCM, FANCN, FANCO, FANCP and BRCA2 (previously known as FANCD1) [84]. Damaging mutation in any of these genes inhibits the efficacy of the core FA protein complex and limits its ability to act in DNA repair. The FA pathway is involved in DNA repair when the two strands of DNA are incorrectly joined together, a process also known as interstrand cross linking [84]. The FA pathway also affects many other pathways, such as nucleotide excision repair and homologous recombination [84].

Genetic mutations in four DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) and the EPCAM gene have been implicated in Lynch syndrome [85]. Defective *MMR* genes allow continuous insertion and deletion mutations in regions of DNA known as microsatellites, leading to a state of microsatellite instability [85]. Mutated microsatellites are involved in tumour initiation and progression and prevent the proper repair of DNA prior to cell division, allowing abnormal cells to divide and thus increasing the risk for cancer [85].

Xeroderma pigmentosum (XP) may be caused by genetic mutations in eight genes, which produce the following enzymes: XPA, XPB, XPC, XPD, XPE, XPF, XPG and Pol η [84]. XPA and XPF are nucleotide excision repair enzymes that repair UV light-damaged DNA, and faulty proteins will allow the build-up of mutations caused by UV light [84]. Pol η is a polymerase which replicates UV light-damaged DNA, and mutations in this gene will produce a faulty pol η enzyme that cannot replicate DNA with UV light damage [84].

Immune System and Host Response

The immune system plays a key role in the progression of head and neck cancer, and a greater understanding of its contribution will lead to better therapies and improved patient outcome.

Immune surveillance is the destruction of nascent cancer cells by the immune system before tumour formation can occur [57]. Immune system derangements or alterations in transformed cells may allow immune escape that allows the cancer to become manifest [57]. There are global alterations in the functional state of the immune system, as evidenced by changes in serum cytokines, chemokines and other immune-related biomarkers in cancer patients. Cancer cells evade the immune system by two primary mechanisms: by reducing their innate immunogenicity or by suppressing the immune response. Tumour cells can reduce T-cell-mediated recognition by altering HLA class I expression, and it has been noted that some tumour cells have a complete

loss of HLA expression due to defects in b2-microglobulin expression or function [57]. Alternatively, chromosomal defects in HLA-encoding genes themselves can cause selective loss of HLA expression, and this process has been noted in approximately 50% of head and neck squamous cell carcinomas and is correlated with poor prognosis in oesophageal and laryngeal squamous cell cancers [57].

In head and neck cancer, circulating serum antibodies have been found against p53, MUC1, p40, p73 and HPV E6 and E7 [57]. High postoperative levels of anti-p53 antibody have been correlated with poor prognosis. Of unclear significance is whether the levels of circulating antibody have any correlation with clinical outcome and whether the reported increase in frequency of IgE subtype immunoglobulins in head and neck cancer is of any importance. In developed head and neck cancers, an endogenous host immune response is prognostic, as has been demonstrated for multiple tumour types: T-cell infiltration of both CD4+ and CD8+ populations have been found to be prognostic in tonsillar and base of tongue SCCs, lymph node-infiltrating CD8+ T cells as well as CD20+ B cells were found to be prognostic in both oropharyngeal and hypopharyngeal cancers, and peritumoural CD8+ T cells in oral cancer have been found to be associated with lymph node metastases, tumour size and clinical stage [86].

In other cancers, there is ample expression of HLA and tumour antigen but without recognition by T cells [57]. Because HLA loss variants are killed by NK cells, one proposed explanation for the lack of NK cell killing is that cancer cells possess defects in their antigen presentation machinery (APM) as this would reduce selectively tumour antigen-HLA peptide completely without reduction in overall surface HLA density [57]. Antigenic peptides are transported to the endoplasmic reticulum by the transporter associated with antigen processing (TAP) where they are associated with HLA class I heavy chains by tapasin [57]. Thus, HNSCC cells that express HLA I and whole tumour antigen can evade T-cell recognition through decreased expression of LMP2, TAP1, TAP2 and tapasin [57]. In addition to decreased expression of HLA, HNSCC tumour cells express Fas ligand which can interact with Fas and transduce a powerful apoptotic signal to activated T cells allowing immune evasion by eliminating tumour-infiltrating T lymphocytes [57]. As mentioned, decreased expression of HLA molecules is protective against T cells but increases NK cell-mediated cytolysis, as the absence of HLA removes a key inhibitory signal for NK cells, and tumour cells must therefore employ multiple mechanisms to suppress NK cell-mediated antitumour immunity. MICA, a ligand of NKG2D in NK and T cells, can be released in a soluble form to act as a competitive antagonist [57]. Known to be produced by tumour cells are immune-suppressive cytokines and other molecules such as IL-10, TGF-b, IL-6, PGE, VEGF and GM-CSF [57]. IL-10 reduces activation of cytotoxic T cells and has been correlated with advanced-stage head and neck cancer; TGF-b suppresses T-cell and NK activation and is a key cytokine in the differentiation of regulator T cells; TGF-b production is increased in preneoplastic oral cavity lesions and promotes angiogenesis and a protumourigenic microenvironment linking it to early tumour formation. Transcription factors such as NFk(kappa)B (nuclear factor kappa-light-chain-enhancer of activated B cells) and STAT3 (signal transducers and activators of transcription), which are usually dysregulated in tumour-promoting inflammatory states in response to cytokine

stimuli, are aberrantly activated in tumour cells and are intensively studied as possible targets for therapeutic intervention [57]. Tumours themselves produce cytokines such as TGF-b(beta), IL-6 and IL-10, which suppress cell-mediated antitumour immunity [57]. In response to inflammatory stimuli, head and neck cancer cells also express receptors which are involved in lymphocyte and dendritic cell migration [57]. Expression of these receptors by tumour cells, such as CCR7 and CXCR4, constitutes immune exploitation of established signals intended for immune cells and has been associated with tumour invasion, metastasis and cell survival, leading to treatment resistance [57].

The role of the immune system in the development of all head and neck malignancies is evident in virally mediated cancers such as HPV-associated oropharyngeal tumours and EBV-associated nasopharyngeal cancers [86]. While both EBV and oral HPV infections are on the rise, nasopharyngeal and oropharyngeal cancers develop less frequently, and this is presumably due to the failure of the immune system to remove these oncogenic infections [86]. The immune checkpoint ligand programmed cell death (PD)-L1 has been identified in tonsillar crypts irrespective of HPV infection, and PD-1+-infiltrating lymphocytes are found in both chronic tonsillitis and HPV-associated oropharyngeal tumours; once HPV infection is established, multiple immune-inhibitory mechanisms, including activation of the PD-1/PD-L1 axis, may contribute to T-cell dysfunction and exhaustion [86]. Head and neck cancers not associated with HPV infection likely also co-opt immune regulatory mechanisms to facilitate their progression. Increased PD-L1 expression similarly has been detected in tobacco- and alcohol-induced SCC of the head and neck as well as other virally mediated tumours, including nasopharyngeal carcinoma and natural killer T (NKT)-cell lymphoma [86].

Premalignant lesions may be immunogenic and targetable with immunologic therapies to prevent progression to malignancy. For example, increased PD-L1 expression has been demonstrated on actinic cheilitis as well as respiratory papilloma lesions which can progress to larynx cancer. Although not necessarily indicative of a premalignant condition, a systemic antibody response directed against the oncogenic HPV E6 and E7 proteins has been demonstrated to be highly specific for the eventual diagnosis of oropharyngeal cancer, and this includes antibody responses that predate oropharyngeal cancer diagnosis by several years [86]. These antibody titers could be used to identify those at highest risk for inclusion in surveillance protocols, and in a similar manner, IgA antibody responses directed against EBV antigens have also been investigated for their ability to aid in the diagnosis of nasopharyngeal cancers [86].

Malignant Transformation

Genetic Alterations in Oral Cancer

The typical solid cancer harbours between 33 and 66 somatic mutations likely to produce changes in protein expression, and OSCC is at the high end of this spectrum with 66 mutations, although not as dramatic as melanoma or lung cancer which involve potent mutagens (approx. 150) or MSI colorectal tumours displaying

deficiencies in DNA damage repair genes [87]. It has been estimated that each driver mutation will increase the likelihood of cell survival by just 0.4% but that this growth advantage will become important with increasing mutational burden and time [87].

Large-scale sequencing efforts such as the Cancer Genome Atlas (TCGA) have allowed a greater understanding of the cancer genome and permitted classification of OSCCs that may be histologically similar on the basis of their genetic differences [47]. Table 3.2 (adapted from Kang et al. [88]) summarises the gene mutations

Table 3.2 Gene mutations in head and neck squamous cell carcinoma identified by The Cancer Genome Atlas

Mutated gene	Protein	Cancer gene class	% Tumours with mutation in TCGA (n = 279)	Hallmark involvement
*TP53**	p53	TSG	72	Evasion of growth suppressors and apoptosis, proliferative signalling
*NOTCH1**	Notch1	TSG	19	Evasion of growth suppressors and apoptosis, proliferative signalling
*CDKN2A**	p16 and p14arf	TSG	22	Proliferative signalling, apoptosis (p14arf)
*PIK3CA**	p110a	Onco-gene	21	Proliferative signalling
FBXW7	F-box/WD repeat-containing protein 7	TSG	5	Targets cyclin E for degradation, controls Notch1 stability
*HRAS**	p21, H-Ras	Onco-gene	4	Growth factor signalling, proliferation
SYNE1	Syne-1	NA	18	Cytoskeleton, centrosome migration
*FAT1**	Proto-cadherin Fat1	NA	23	Cadherin, Wnt signalling
KMT2D (MLL2)	KMT2D	TSG	18	Histone methylation (immortalisation)
*CASP8**	Caspase 8	TSG	9	Apoptosis
PTEN	PTEN	TSG	2	Protects against genomic instability, controls proliferation, apoptosis
NSD1		NA	10	Transcription factor
KMT2C (MLL3)	Lysine methyltransferase	TSG	8	Histone methylation (immortalisation)
EP300	P300	TSG	7	Regulates proliferation differentiation

The table summarises 14 of the most prevalent gene mutations identified in a large-scale screen of 279 head and neck cancers performed by The Cancer Genome Atlas consortium [47, 114]. As well as the mutated gene and its prevalence in the TCGA cohort, the table includes the protein coded for by the gene and where the gene/protein fits in terms of cancer hallmarks and classifies genes as either tumour suppressor genes (TSG), oncogenes or neither (NA). Genes highlighted with an asterisk have been demonstrated to occur specifically in OSCC, although generally present at a lower prevalence than in the entire HNSCC TCGA cohort [163, 164]

found by TCGA [47] and outlines the functions and cancer hallmark associations of the proteins they code for.

TCGA has provided an unprecedented level of characterisation of the mutational landscape of advanced cancers, which has driven novel treatments and improved patient care. However, it is recognised that the most effective interventions are those that target cancer at an early stage, prior to malignant transformation. In order to do this effectively, there has been a recent call for a concerted research effort towards the development of a 'Pre-Cancer Genome Atlas' (PGCA) [89, 90]. The PCGA calls for the 'comprehensive genetic profiling of premalignant lesions' performed over time, with associated histological results and outcomes [89]. This information, when applied to oral premalignant lesions, would allow the production of a catalogue of driver genes for OSCC. This in turn would allow more accurate stratification of low- and high-risk OPMLs, and personalised treatment as new immuno- and chemotherapeutics becomes available. This approach underpins our current research efforts, but significant advances in this domain will require international collaboration and scientific will to tackle the oral premalignant disease problem, as this represents an ideal model for understanding pre-malignancy while concurrently driving the PCGA agenda. The recent 'Erlotinib Prevention of Oral Cancer' (EPOC) trial provided the first example of 'high-risk' OPML being treated chemotherapeutically in an attempt to prevent progression to OSCC, and while no benefit was shown for the treatment drug, it did provide a valuable proof of concept approach [91].

The molecular characterisation of oral carcinogenesis has not been fully defined; however, it has been well-documented in the case of colon cancer. In this cancer, the first 'gatekeeper' mutation is typically in the tumour suppressor gene *APC* (adenomatous polyposis coli), which provides a selective advantage to the cell allowing it to outcompete its surroundings and start to produce a small, slow-growing adenoma [92]. A second mutation is characteristically in the gene *KRAS*, which produces an important signalling molecule and allows cells carrying both mutations to proliferate. Further mutations in genes such as *PIK3CA*, *SMAD4* and *TP53* give the cells the capacity to invade basement membrane and metastasise [87].

Biomarkers of Malignant Transformation

Oral cancer is frequently preceded by oral potentially malignant lesions (OPMLs); however, a comparatively small proportion of OSCCs, between 5% and 15%, will undergo malignant transformation [16]. Histopathology is currently the gold standard to identify and monitor OPMLs for the risk of malignant progression; however, given the invasiveness and variability of this technique, there is great interest in the identification of biomarkers that are able to segregate progressive from non-progressive OPML.

A biomarker can be defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or

pharmacologic responses to a therapeutic intervention' [93]. Biomarkers can be prognostic (information of prognosis and the potential of recurrence), diagnostic (detection) or predictive (predict response to treatment). We will focus on biomarkers that are useful in both identifying OSCC and predicting which OPMLs are likely to convert to OSCC. We will include markers of genomic instability, gene and protein biomarkers and epigenetic modulators.

Measures of Genomic Instability (CNV and LOH)

Genomic instability underlies carcinogenesis and malignant transformation, and assessment of genomic integrity has been utilised as a biomarker of malignant transformation. Changes in DNA content/ploidy, copy number variation and loss of heterozygosity are all manifestations of genomic instability that occur over the process of carcinogenesis, and particular patterns of these can be used to identify malignant transformation [18].

DNA content/ploidy can be used as a surrogate marker of genomic damage and is measured by flow or image cytology. Tobacco smoking induces aneuploidy in normal oral mucosa [94], and leukoplakia has been correlated with changes in ploidy [95].

Copy number variation describes abnormal duplication or insertions across the genome and is a measure of genomic instability. Characteristic copy number variations of −8p/+3q/+8q have been identified in oral cancer but are also present in oral epithelial dysplasia suggesting they may be an early step in malignant transformation. HNSCC in smokers displays a high prevalence of copy number changes particularly amplifications of 3q26/28 and 11q13/22 [47]. A recent study investigated copy number changes in tumour margins and was able to correlate changes in chromosomes 1 and 7 with tumour recurrence. Copy number changes in at least one tumour margin resulted in a higher risk of local recurrence and a decreased 5-year recurrence-free survival rate (47.1% vs. 88.9%) [96].

Loss of heterozygosity (LOH) describes a loss of genomic material, in a somatic cell, at a heterozygous region so that only one copy of a gene remains. In the case of cancer, where mutations are frequent, this can leave the remaining copy of a tumour suppressor gene, for example, vulnerable to inactivating mutation.

OSCC is characterised by chromosomal gains at 1q, 3q, 5p, 7p, 8q, 9q, 11q, 14q and 18p and losses at 3p, 4p, 4q, 5q, 8p, 9p, 10p, 11q, 13q, 17p, 18q and 21q [97, 98]. Malignant transformation has been correlated with LOH 3p and 9p (as well as 8p, 11q, 13q and 17p), and the transition to invasive carcinoma has been associated with LOH (6p, 8p, 4q 26–28, 10q23). Leukoplakia with LOH at 3p and 9q display a 3.8-fold increase in malignant transformation, and this is present in approximately 50% of leukoplakia [99]. A further study found LOH at 9p21 and/or 3p14 in 51% of leukoplakia, and 37% of these patients developed HNSCC compared with only 6% of leukoplakia lacking LOH in these regions [100]. When there is additional LOH at the 4q, 8p, 11q, 13q and 17p loci, the increased risk of malignant transformation increases to 33-fold [101].

The EPOC trial, while not finding efficacy for treatment, did validate LOH as a marker of oral cancer risk. This trial investigated whether the EGRF inhibitor erlotinib could reduce malignant transformation in high-risk OPMLs, defined as OPMLs displaying LOH at either 3p14 or 9p21 with a history of oral cancer or LOH at 3p14 and/or 9p21 plus one other chromosomal site if there was no history of oral cancer [91]. In this cohort, individuals with LOH negative OPML were significantly less likely to progress to cancer, with 13% developing cancer over 3 years, compared with 26% of individuals with LOH positive OPMLs [91].

Modifiers of Expression: lncRNA and miRNA

Alterations to protein coding sequences encompass only part of the complexity in cancer. Cancer-related changes in microRNA (miRNA), long non-coding RNA (lncRNA), short nucleolar RNA (snoRNA) and epigenetic modification have been demonstrated to influence oral carcinogenesis and malignant transformation [102]. miRNAs are short noncoding RNAs 21–23 nucleotides in length that regulate the expression of 30–60% of protein-coding genes and are also able to influence epigenetic remodelling [103]. miRNAs have been demonstrated to act as oncogenes (e.g. miR21) or tumour suppressor genes by influencing the expression of tumour suppressor or oncogenic proteins.

There are several lines of evidence suggesting that miRNAs can be implicated in oral malignant transformation. miR21, miR181b and miR345 have been found to be positive markers of malignant transformation [104]. miR21 is a known oncogenic miRNA that is present in a number of cancer types and has been linked to poor patient prognosis in tongue cancer [105]. The study in tongue cancer also identified miR7 as another candidate oncogene and miR375 and miR-494 as candidate tumour suppressors [105]. Expression of these miRNAs was then assessed in progressive versus non-progressive OPMLs (5-year follow-up), and low levels of miR375 were predictive for malignant transformation [106]. It is thought that miR375 may affect the expression of Survivin via the transcription factor KLF5, thus influencing apoptosis and proliferation and potentially influence invasiveness [107]. Salivary levels of miR31 were recently shown to be an independent risk factor for the progression of OPMLs [108]. Screening of miRNAs in saliva has tremendous promise as a non-invasive strategy for monitoring lesions but has not yet been clinically validated.

lncRNAs are non-coding RNAs of more than 200 nucleotides in length. They are known to silence miRNAs and modulate the expression and cellular localisation of proteins [109, 110]. Several lncRNAs have been implicated in cancer. A study in OSCC found that the lncRNA HOTAIR was upregulated (metastasis) and *GAS5* (growth arrest-specific transcript 5) and *MEG-3* (maternally expressed gene 3, tumour suppressor) were downregulated compared with normal tissue [111]. This study also identified two novel tumour-suppressive lncRNAs that were downregulated in OSCC—*lnc-LCE5A-1* and *lnc-KCTD6-3*. These were able to reduce proliferation and migration as well as gene expression associated with stem cells and the epithelial-mesenchymal transition (EMT) when overexpressed in HNSCC cell

lines. A recent discovery study identified 728 lncRNAs that were expressed differently in HNSCC versus normal adjacent tissues, including some that had been previously implicated in cancer [112]. This study provides a pool of differentially expressed lncRNA that can be used to further investigate the use of lncRNA as biomarkers in OSCC.

Gene and Protein Biomarkers by Hallmark of Cancer Characteristics

There is a plethora of gene and protein biomarkers that have the potential to identify and/or predict malignant transformation. They have been divided into functional groups by cancer hallmarks and are summarised in Fig. 3.2.

Sustained Proliferative Signalling and Evasion of Growth Suppressors and Apoptosis

In normal cells, signalling, typically involving kinase receptor pathways and growth factors, regulates progression through the cell cycle and cell growth. Cancer cells are able to circumvent this control and thus maintain proliferation in a number of ways. This may involve the direct production of growth factors by cancer cells, the production of signals stimulating surrounding cells to produce growth factors, the overexpression of receptor proteins at the cell surface rendering the cell hyperresponsive to proliferative signalling or the activation of downstream mediators of the signalling pathway [18]. In oral cancer, the signalling molecules EGFR, FGFR, MET, PIK3CK and CCND1 and members of the Wnt pathway (AJUBA, FAT1 and NOTCH1) are important in maintaining the proliferative signalling characteristic of malignant cells. Cancerous cells must also avoid the endogenous suppression of proliferative signalling that operates in normal cells. Typically, this control over cell growth is orchestrated by tumour suppressor proteins that regulate the switch between proliferation and apoptosis/senescence. Tumour suppressor proteins may also induce apoptosis, for example, TP53 acts by inducing apoptosis when DNA damage and chromosomal abnormalities become too great [113]. Apoptosis involves balancing of the pro- and anti-apoptotic members of the B-cell lymphoma 2 (Bcl2) family. TP53 is one of the classic tumour suppressor proteins and is mutated in 69.8% of HNSCC in TCGA cohort and 42% of samples in the Pan Cancer cohort [47, 114]. Interestingly, elephants, which have a low rate of cancer compared to other mammals, were recently found to carry up to 20 copies of p53, and elephant cells show increased rates of apoptosis after exposure to a mutagenic stimuli [44].

Mutations in the epidermal growth factor receptor (EGFR) are present in 15% of HPV-negative and 8% of HPV-positive HNSCC [47]. Most HNSCC display high EGFR expression compared with normal tissue and high expression of EGFR as well as its ligand transforming growth factor-alpha is associated with poor progno-

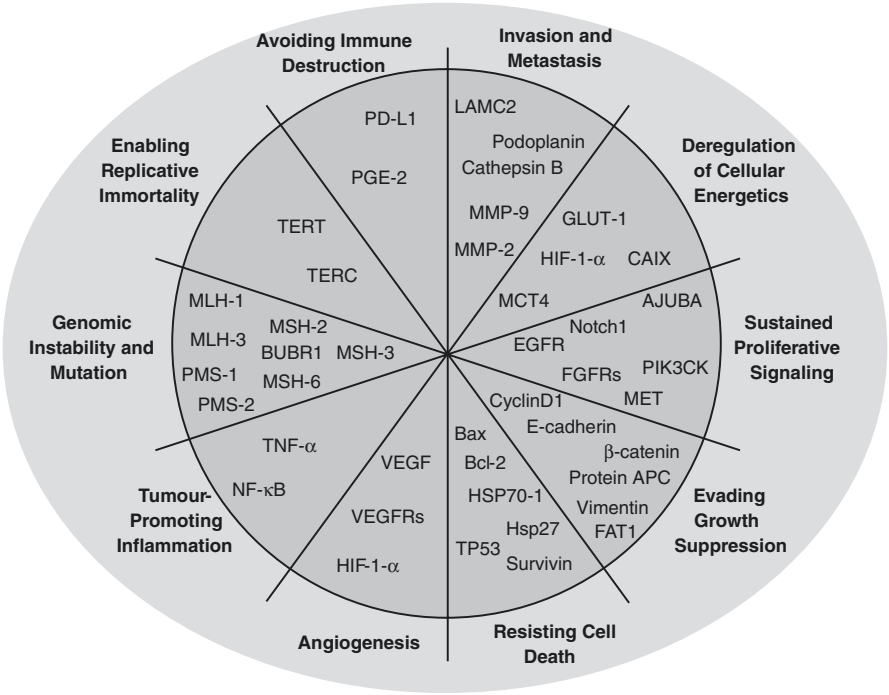

Fig. 3.2 Gene and protein biomarkers divided by hallmark of cancer characteristic. The identification of sensitive and accurate biomarkers of oral malignant transformation is a powerful tool in the early identification of OSCC. The figure displays a collection of biomarkers, divided into their hallmarks of cancer characteristics that have been identified as potential biomarkers in OPML and/or OSCC. Full names and abbreviations are provided below. Adenomatous polyposis coli protein (**Protein APC**); LIM domain-containing protein ajuba (**AJUBA**); apoptosis regulator BAX (**Bax**), also known as Bcl-2-like protein 4 (Bcl2-L-4); apoptosis regulator Bcl-2 (**Bcl-2**); MAD3/BUB1-related protein kinase (**BUBR1**), also known as mitotic checkpoint serine/threonine-protein kinase BUB1 beta (BUB1-β); catenin beta-1 (**β-catenin**); carbonic anhydrase 9 (**CAIX** or CA9); **Cathepsin B**; G1/S-specific cyclin-D1 (**Cyclin D1**); epithelial cadherin (**E-cadherin**), also known as Cadherin 1; epidermal growth factor receptor (**EGFR**); protocadherin Fat 1 (**FAT1**); fibroblast growth factor receptors 1, 2 and 3 (**FGFR-1**, **FGFR-2** and **FGFR-3**); glucose transporter type 1 (**GLUT-1**), also known as solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1); heat shock 70 kDa protein 1 (**HSP70-1**), also known as heat shock 70 kDa protein 1A; heat-shock protein beta-1 (**HspB1**), also known as heat shock 27 kDa protein (**Hsp27**); hypoxia-inducible factor 1-alpha (**HIF-1-α**); laminin subunit gamma 2 (**LAMC2**); monocarboxylate transporter 4 (**MCT4**), also known as solute carrier family 16 member 3 (SLC16A3); tyrosine-protein kinase Met (**MET**), also known as hepatocyte growth factor receptor (HGF receptor); matrix metalloprotease 2 and 9 (**MMP-2** and **MMP-9**); MutL protein homolog 1 and 3 (**MLH1** and **MLH3**), also known as DNA mismatch repair proteins Mlh1 and Mlh3; MutS protein homolog 2, 3 and 6 (**MSH2**, **MSH3** and **MSH6**), also known as DNA mismatch repair protein Msh2 (hMSH2, hMSH3 and hMSH6); neurogenic locus notch homolog protein 1 (**Notch 1**); nuclear factor NF-kappa-B p65 subunit (**NF-κB**), also known as transcription factor p65; apoptosis inhibitor survivin (**Survivin**), also known as baculoviral IAP repeat-containing protein 5 (BIRC5); telomerase RNA template component (**TERC**); telomerase reverse transcriptase (**TERT**); tumour necrosis factor alpha (**TNF-α**); cellular tumour antigen p53 (**TP53**); programmed cell death 1 ligand 1 (**PD-L1**); prostaglandin E_2 (**PGE-2**), produced by the protein cyclooxygenase 1 or 2 (COX-1, COX-2); **Podoplanin**; p110α catalytic subunit of phosphinositol-3-kinase (**PIK3CA**); post-meiotic segregation increased protein homolog 1 and 2 (**PMS1** and **PMS2**); vascular epidermal growth factor a (**VEGF-A** or **VEGF**); **Vimentin**; vascular endothelial growth factor receptor 1, 2 and 3 (**VEGFR-1**, **VEGFR-2** and **VEGF-3**)

sis [115]. A recent study found that abnormal EGRF gene copy number was a positive predictor for malignant transformation of OPMD [116]. The EPOC trial also found that increased EGRF gene copy number was associated with reduced cancer-free survival in OPMLs and correlated with LOH [91].

Fibroblast growth factor receptors (FGFRs) have diverse functions. Ligand binding triggers downstream signalling that influences differentiation, proliferation and angiogenesis. Gain of function mutations in receptors and ligands have been reported in other cancer types (reviewed in [117]). FGFR1 displays genetic alteration in 10% of HPV-negative HNSCC and FGFRs 2, 3 and 4 in 2% or less. Eleven percentage of HPV-positive HNSCC display alterations in FGFR3 and 3% mutations in FGFR3 [47]. Immunohistochemical staining of FGFR-2 and its ligand FGF-2 was recently performed in OPML and OSCC samples, and their expression found to be a positive predictor of malignant transformation [118]. FGFR-3 expression was present in 48% and FGFR-4 expression in 41% of OSCCs [119, 120].

MET (hepatocyte growth factor receptor) is a proto-oncogene that signals from the extracellular matrix to the cytoplasm. Once it has bound to hepatocyte growth factor, it has pro-survival properties, including roles in migration, invasion and angiogenesis in cancer. MET or its ligand is expressed in approximately 80% of HNSCC, despite only being mutated or amplified in a relatively low number of HNSCCs in TCGA [88, 121].

CCND1 is the gene coding for the cyclin D1 protein. It is the regulatory component of the CDK4/cyclinD1 complex which regulates the cell cycle from G1/S transition. CyclinD1 modulates expression of CDK4 kinase (GeneCards). Twenty-four to 48% of dysplastic OPML had alterations in CCND1 [122]. TCGA found that the 11q amplicon, which contains the CCND1 gene, is frequently altered in HPV-positive HNSCC. Approximately 20% of HNSCC display mutations in CCND1 [114]. Expression of cyclinD1 measured by IHC correlated with malignant progression of leukoplakia and erythroplakia [123, 124]. CyclinD1 is believed to be upregulated early in oral carcinogenesis and can be detected in saliva in individuals with oral cancer [50].

The PIK3CK gene encodes the p110 alpha protein, which is the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). PI3K is part of the AKT signalling pathway, which regulates cell proliferation, migration and survival. In oral cancer, PIK3CK is an oncogene, and mutations in PIK3CK are common, particularly in HPV-positive tumours. Approximately 21% of OSCCs display alterations in PIK3CA, and mutations activating PIK3CA were prevalent in a sub-group of OSCCs that displayed improved clinical outcomes [47, 114].

Genes of the Wnt pathway including AJUBA, FAT1 and Notch1 are important in regulating cellular proliferation and are frequently mutated in oral cancer with Notch1 mutations present in 19.3% of HNSCC [114]. Notch1 mutations are also present in leukoplakia, and around 60% of these mutations are also present in OSCC, suggesting that Notch1 mutation is an early event in carcinogenesis [125]. Inactivation of AJUBA, FAT1 and Notch1 causes deregulation of cellular polarity and differentiation and may contribute to malignant transformation.

Other members of the Wnt signalling pathway include E-cadherin, β-catenin, APC and Vimentin, which have shown to be potential markers of malignant transformation [126] and LGR5 which shows increasing expression from OED to OSCC [127].

The cyclin-dependent kinase inhibitor 2A (CDKN2A) gene codes for the p16 tumour suppressor, as well as producing several splice variants. It acts by regulating the cell cycle and is a tumour suppressor. CDKN2A is mutated in 21.3% of HNSCC [114]. In HPV-negative HNSCC, p16 expression is reduced favouring cellular proliferation [128, 129]. Infection of the oral mucosa with high-risk HPV induces overexpression of p16 in OPML and OSCC so it can be utilised as a surrogate biomarker for HPV infection, although this can lead to false positives if used alone [128–130].

Heat-shock proteins are expressed in response to stress and may inhibit apoptosis. HSP70 and HSP27 can act as markers of epithelial dysplasia in leukoplakia [131]. Apoptotic pathway members Bcl-2, Bax and Survivin have been shown to display altered expression in oral cancer and precancer [132, 133].

Replicative Immortality

Normal cells can only go through a limited number of cycles of cell division before they enter a state of senescence or crisis and subsequent apoptosis. These processes are mediated via telomeres, which wrap around the ends of chromosomes, protecting them from damage. Each cycle of cell division shortens the telomeres until they are no longer able to protect the chromosome from damage. Occasionally, cells may emerge from crisis with the capacity to undergo unlimited proliferation and are termed immortalised. Telomerase is a ribonucleoprotein which consists of two components: telomerase reverse transcriptase (TERT), the enzyme component and telomerase RNA component (TERC), which acts as a template for the telomere repeat TTAGGG which is added to telomere ends. Immortalised cells (including cancer cells) are able to prevent telomeres from eroding and express much more telomerase (which lengthens the telomere DNA) than normal cells [134, 135]. Telomerase reverse transcriptase (TERT) is one of the protein subunits of telomerase and has both enzymatic activity and is involved in proliferative signalling via the Wnt pathway. TERT mediates the elongation of telomeres, promotes cell immortalisation and has also been shown to increase invasiveness [136]. TERT expression is not present in normal or mildly dysplastic oral mucosa but is present in moderately dysplastic tissues as well as those displaying severe dysplasia and OSCC [137]. A recent study combining morphological assessment and detection of hTERC (the RNA component of telomerase) via FISH found that acquisition of the hTERC gene predicted malignant progression in OPMLs [138]. When telomerase activation was measured in HNSCC and OPML, the levels were comparable (78% and 85%, respectively) but had increased by 25–32% compared with normal, adjacent tissue [139]. Reduced telomere length is found in tissues adjacent to CIS, and orthokeratotic leukoplakia is associated with particularly short telomeres [51, 140].

Together, these studies suggest that cellular immortalisation occurs relatively early in oral carcinogenesis.

Invasion and Metastasis

Invasion and metastasis in oral cancer are driven by the epithelial to mesenchymal transition (EMT), a developmental program that involves the conversion of epithelial cells to a mesenchymal phenotype. This phenotypic change involves a loss of cellular polarity, a switch to a spindle shape, increased motility and resistance to apoptosis. EMT is also associated with increased expression of enzymes capable of digesting extracellular matrix to facilitate invasion.

Matrix metalloproteases are a family of zinc metalloenzymes with the capacity to digest a range of components of the extracellular matrix, including gelatin, collagen, stromelysin and membrane components. MMP-2 and MMP-9 remodel extracellular matrix and are important in mediating invasion and metastasis in OSCC. In a study of tongue cancer, MMP-2 and MMP9 were found to increase expression as carcinogenesis progressed, with rare expression in normal epithelium, expression in approximately 40% of non-metastatic tongue SCC and expression in 70–80% of metastatic SCC [141]. While normally associated with the later stages of carcinogenesis, salivary levels of MMP-9 are increased in OPMLs compared with healthy controls and then further increased in OSCCs [142]. A recent meta-analysis concluded that MMP-9 was a viable biomarker to predict malignant progression [56].

Podoplanin (coded by the PDPN gene) is a transmembrane protein that is upregulated during epithelial-mesenchymal transition and has roles in migration in many human cancers [143]. Podoplanin is a useful marker of malignant transformation in erythroplakia [144], and a recent paper has suggested that the risk of transformation in OPMLs is enhanced threefold if podoplanin is co-expressed with ELAV like RNA-binding protein 1 (HuR) [145]. Laminin subunit gamma 2 (LAMC2) is an extracellular matrix glycoprotein and a contributor to the breakdown of the basement membrane in oral cancer. When expressed in leukoplakia, LAMC2 predicts imminent malignant transformation [146]. Cathepsin D and B forms have also been correlated with invasion and metastasis in OSCC [147].

Avoiding Immune Destruction

Patients with HNSCC display immune suppression with reductions in antigen presentation, reduced lymphocyte counts and inhibited NK cell activity [97]. Evidence for this includes cases of human-associated transplant cancer, where cancer develops in immunosuppressed recipients when no longer held in check by the healthy immune system of the donors [148].

Tumour-associated macrophages (TAMs) have a demonstrated role in cancer with the M2 phenotype considered to be pro-inflammatory and tumour promoting and the M1 phenotype protective. Premalignant lesions have been shown to contain

M1 TAMs and OSCC primarily the M2 phenotypes [149, 150]. This suggests that TAM phenotype can be used as a marker of malignant transformation. IL-37 acts to suppress the innate immune system and could provide a potential marker for malignant transformation in leukoplakia as there was a correlation between the degree of dysplasia and IL-37 protein expression [151].

In the healthy immune system, CD8+ T cells and CD4+ helper T (Th)1 cells inhibit cancer formation by secreting interferon gamma (IFN-γ) and cytotoxins [126]. A recent study provided evidence that the presence of CD3+ T cells may protect against malignant transformation. The dysplastic leukoplakia that progressed to OSCC had significantly less CD3+ T cells than those that did not [152].

OPMLs have been shown to display increasing immune cell infiltration as they progress through grades of dysplasia and into carcinoma [150]. Ohman et al. demonstrated increased numbers of Langerhans cells and T cells in dysplastic cell compartments and OSCC [153]. OPMLs may also start to induce inflammation and modulate the immune system before they undergo malignant transformation. In a mouse model, cells derived from premalignant oral lesions secreted substances that induced T-cell activation and expression of IL-2 receptor [102]. Programmed death 1 (PD-1) and its receptor (PD-1R) have been implicated in tumour escape and are expressed in premalignant as well as malignant tissues [154]. A recent immunohistochemical study found that 29% of OSCC lesions express PD-L1 and 83% contain PD-1 positive lymphocytes [155].

Genomic Instability and Mutation

Genomic rearrangements and mutations have been well characterised in OSCC and to a lesser extent in OPMLs. There are a number of systems that maintain genomic stability in normal cells, including the DNA mismatch repair system and the mitotic spindle checkpoint.

The DNA mismatch repair system in humans consists of MutL protein homolog 1 and 3 (MSH1 and MSH3), MutS protein homolog 2, 3 and 6 (MSH2, MSH3 and MSH6) and post-meiotic segregation increased 1 and 2 (PMS1 and PMS2), and they work sequentially to repair DNA mismatches [3]. MSH2 heterodimerises with MSH6 to form MutS-alpha, the predominant form of MutS in humans, which recognises mismatches. MLH1 and PMS2 heterodimerise to form MutL-alpha, again the primary form in humans, which links MutS-mediated mismatch repair with downstream activators [52]. Deficiencies in this system have been clearly implicated in non-hereditary colon cancer [156], but there is evidence that these are also important in OPML and OSCC.

OLP display decreased protein expression of MSH2 in comparison to normal tissues, and MLH1 protein expression can be correlated with the degree of dysplasia in leukoplakia [157]. A recent study assessed protein expression of all the major MMR components across the spectrum of OED and OSCC and found that MLH1, PMS2 and MSH2 were reduced in malignant and premalignant tissues compared with normal [158]. The dimerisation components (i.e. MSH2/MSH6 and MLH1/

PMS2) correlated with each other, and the specific loss of MSH6 in the basal layers was shown to be a useful diagnostic marker for carcinoma in situ [159].

BUBR1, one of two putative vertebrate homologs of the yeast spindle checkpoint protein BUB1, has two important roles in mitosis, with the C-terminal kinase domain regulating chromosome movement and the N-terminus involved in checkpoint, pausing anaphase until all chromosomes are properly attached to the mitotic spindle [160]. BUBR1 is overexpressed in a subset of OSCC cases [161] and promotes cellular mobility and invasion via MMPs in OSCC cell lines [162]. Immunohistochemical evidence suggests that BUBR1 is expressed in premalignant lesions and may act as a biomarker for oral carcinogenesis [63].

Conclusion

OSCC is frequently identified at an advanced stage, which limits treatment options and leads to increased patient mortality and morbidity. A better understanding of the molecular changes that drive oral carcinogenesis has the potential to deliver improved screening, treatment options and patient outcomes.

Oral carcinogenesis can be understood using clinical, histopathological and molecular models, which describe the physical manifestation of the lesion, the alterations in tissue architecture and cell cytology within the lesion and the genetic alterations leading to dysfunctional protein expression/function within the cells of the lesion. Our understanding of oral carcinogenesis has been significantly enhanced by the development of animal models which utilise carcinogenic agents applied to rats, mice or hamsters and mimic the development of human oral cancer. These, along with population-based studies, have been useful in unravelling the impact of various risk factors on the development of oral cancer, including smoking and alcohol consumption. Genetic conditions that predispose individuals to the development of cancer have also provided insight into oral carcinogenesis, particularly the importance of DNA repair systems in protecting against cancer. Large-scale sequencing efforts have provided a wealth of information about the genetic alterations that occur in cancerous cells and allowed for a more meaningful segregation of cancer subtypes and personalisation of treatment.

The development of oral cancer can be understood as the acquisition of mutations that allow the development of cancer hallmarks or properties that allow cancer cells to survive, proliferate and metastasise. Biomarkers are measurable characteristics that can be used to identify OSCC and predict which OPML are likely to transform to cancer. These include markers of genomic instability, the presence of expression modifiers such as miRNA and a range of gene and protein markers. We have divided the gene and protein markers into groupings by functional capability and assessed their value as markers of current or future malignant transformation. Changes in protein expression of many of these biomarkers have been identified in OPMLs, suggesting that they have potential utility as predictors of malignant transformation, although much more work is required to realise these in clinical practice.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Johnson NW, Jayasekara P, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. Periodontol 2000. 2011;57(1):19–37.
3. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet. 2008;371(9625):1695–709.
4. Speight PM, Epstein J, Kujan O, Lingen MW, Nagao T, Ranganathan K, Vargas P. Screening for oral cancer-a perspective from the Global Oral Cancer Forum. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123:680.
5. Winning TA, Townsend GC. Oral mucosal embryology and histology. Clin Dermatol. 2000;18(5):499–511.
6. Garant PR. Oral cells and tissues. Chicago, IL: Quintessence Pub. Co.; 2003.
7. Berkovitz BKB, Holland GR, Moxham BJ. Oral anatomy, histology and embryology. Edinburgh: Mosby; 2014.
8. Squier CA, Kremer MJ. Biology of oral mucosa and esophagus. J Natl Cancer Inst Monogr. 2001;(29):7–15.
9. Lehrer MS, Sun TT, Lavker RM. Strategies of epithelial repair: modulation of stem cell and transit amplifying cell proliferation. J Cell Sci. 1998;111(Pt 19):2867–75.
10. Hume WJ, Potten CS. Proliferative units in stratified squamous epithelium. Clin Exp Dermatol. 1983;8(1):95–106.
11. Michcik A, Cichorek M, Daca A, Chomik P, Wojcik S, Zawrocki A, Wlodarkiewicz A. Tobacco smoking alters the number of oral epithelial cells with apoptotic features. Folia Histochem Cytobiol. 2014;52(1):60–8.
12. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma--an update. CA Cancer J Clin. 2015;65(5):401–21.
13. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45(4-5):309–16.
14. Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. J Oncol. 2011;2011:603740.
15. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, Rosenberg PS, Bray F, Gillison ML. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J Clin Oncol. 2013;31(36):4550–9.
16. Dost F, Do L, Farah CS. Lesion evaluation, screening and identification of oral neoplasia study: an assessment of high-risk Australian populations. Community Dent Oral Epidemiol. 2016;44(1):64–75.
17. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70.
18. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
19. Guo T, Califano JA. Molecular biology and immunology of head and neck cancer. Surg Oncol Clin N Am. 2015;24(3):397–407.
20. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer. 1953;6(5):963–8.
21. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev. 2014;8(1):244.
22. Boyle JO, Hakim J, Koch W, van der Riet P, Hruban RH, Roa RA, Correo R, Eby YJ, Ruppert JM, Sidransky D. The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res. 1993;53(19):4477–80.
23. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res. 2003;63(8):1727–30.

24. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37(1):1–10.
25. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36(10):575–80.
26. Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. J Oral Pathol Med. 2016;45(3):155–66.
27. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006;42(10):987–93.
28. Barnes L, Eveson JW, Reichart P, Sidransky D, editors. World Health Organization classification of tumours. Pathology and genetics of head and neck tumours. Lyon: IARC Press; 2005.
29. Karabulut A, Reibel J, Therkildsen MH, Praetorius F, Nielsen HW, Dabelsteen E. Observer variability in the histologic assessment of oral premalignant lesions. J Oral Pathol Med. 1995;24(5):198–200.
30. Dost F, Le Cao K, Ford PJ, Ades C, Farah CS. Malignant transformation of oral epithelial dysplasia: a real-world evaluation of histopathologic grading. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117(3):343–52.
31. Tanaka T, Kojima T, Okumura A, Yoshimi N, Mori H. Alterations of the nucleolar organizer regions during 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in rats. Carcinogenesis. 1991;12(2):329–33.
32. Vered M, Yarom N, Dayan D. 4NQO oral carcinogenesis: animal models, molecular markers and future expectations. Oral Oncol. 2005;41(4):337–9.
33. Vairaktaris E, Spyridonidou S, Papakosta V, Vylliotis A, Lazaris A, Perrea D, Yapijakis C, Patsouris E. The hamster model of sequential oral oncogenesis. Oral Oncol. 2008;44(4):315–24.
34. Salley JJ. Experimental carcinogenesis in the cheek pouch of the Syrian hamster. J Dent Res. 1954;33(2):253–62.
35. Chang KW, Lin SC, Koos S, Pather K, Solt D. p53 and Ha-ras mutations in chemically induced hamster buccal pouch carcinomas. Carcinogenesis. 1996;17(3):595–600.
36. Solt DB. Localization of gamma-glutamyl transpeptidase in hamster buccal pouch epithelium treated with 7,12-dimethylbenz[a]anthracene. J Natl Cancer Inst. 1981;67(1):193–200.
37. Kwong YY, Husain Z, Biswas DK. c-Ha-ras gene mutation and activation precede pathological changes in DMBA-induced in vivo carcinogenesis. Oncogene. 1992;7(8):1481–9.
38. Kohno Y, Patel V, Kim Y, Tsuji T, Chin BR, Sun M, Bruce Donoff R, Kent R, Wong D, Todd R. Apoptosis, proliferation and p12(doc-1) profiles in normal, dysplastic and malignant squamous epithelium of the Syrian hamster cheek pouch model. Oral Oncol. 2002;38(3):274–80.
39. Nagini S, Letchoumy PV, Thangavelu A, Ramachandran C. Of humans and hamsters: a comparative evaluation of carcinogen activation, DNA damage, cell proliferation, apoptosis, invasion, and angiogenesis in oral cancer patients and hamster buccal pouch carcinomas. Oral Oncol. 2009;45(6):e31–7.
40. Todd R, McBride J, Tsuji T, Donoff RB, Nagai M, Chou MY, Chiang T, Wong DT. Deleted in oral cancer-1 (doc-1), a novel oral tumor suppressor gene. FASEB J. 1995;9(13):1362–70.
41. Gordon HM, Kucera G, Salvo R, Boss JM. Tumor necrosis factor induces genes involved in inflammation, cellular and tissue repair, and metabolism in murine fibroblasts. J Immunol. 1992;148(12):4021–7.
42. Cwikla SJ, Tsuji T, McBride J, Wong DT, Todd R. doc-1--mediated apoptosis in malignant hamster oral keratinocytes. J Oral Maxillofac Surg. 2000;58(4):406–14.
43. Tang XH, Osei-Sarfo K, Urvalek AM, Zhang T, Scognamiglio T, Gudas LJ. Combination of bexarotene and the retinoid CD1530 reduces murine oral-cavity carcinogenesis induced by the carcinogen 4-nitroquinoline 1-oxide. Proc Natl Acad Sci U S A. 2014;111(24):8907–12.
44. Abegglen LM, Caulin AF, Chan A, Lee K, Robinson R, Campbell MS, Kiso WK, Schmitt DL, Waddell PJ, Bhaskara S, Jensen ST, Maley CC, Schiffman JD. Potential mechanisms for

cancer resistance in elephants and comparative cellular response to DNA damage in humans. JAMA. 2015;314(17):1850–60.
45. Tanuma J, Hiai H, Shisa H, Hirano M, Semba I, Nagaoka S, Kitano M. Carcinogenesis modifier loci in rat tongue are subject to frequent loss of heterozygosity. Int J Cancer. 2002;102(6):638–42.
46. Foy JP, Tortereau A, Caulin C, Le Texier V, Lavergne E, Thomas E, Chabaud S, Perol D, Lachuer J, Lang W, Hong WK, Goudot P, Lippman SM, Bertolus C, Saintigny P. The dynamics of gene expression changes in a mouse model of oral tumorigenesis may help refine prevention and treatment strategies in patients with oral cancer. Oncotarget. 2016;7(24):35932–45.
47. Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517(7536):576–82.
48. Field JK. The role of oncogenes and tumour-suppressor genes in the aetiology of oral, head and neck squamous cell carcinoma. J R Soc Med. 1995;88(1):35P–9P.
49. Jurel SK, Gupta DS, Singh RD, Singh M, Srivastava S. Genes and oral cancer. Ind J Hum Genet. 2014;20(1):4–9.
50. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, Gavish M, Nagler RM. Salivary analysis of oral cancer biomarkers. Br J Cancer. 2009;101(7):1194–8.
51. Aida J, Kobayashi T, Saku T, Yamaguchi M, Shimomura N, Nakamura K, Ishikawa N, Maruyama S, Cheng J, Poon SS, Sawabe M, Arai T, Takubo K. Short telomeres in an oral precancerous lesion: Q-FISH analysis of leukoplakia. J Oral Pathol Med. 2012;41(5):372–8.
52. Modrich P. Mechanisms in eukaryotic mismatch repair. J Biol Chem. 2006;281(41):30305–9.
53. Nawroz H, van der Riet P, Hruban RH, Koch W, Ruppert JM, Sidransky D. Allelotype of head and neck squamous cell carcinoma. Cancer Res. 1994;54(5):1152–5.
54. Woodford D, Johnson SD, De Costa AM, Young MR. An Inflammatory cytokine milieu is prominent in premalignant oral lesions, but subsides when lesions progress to squamous cell carcinoma. J Clin Cell Immunol. 2014;5(3):pii: 230.
55. Kujan O, Oliver R, Roz L, Sozzi G, Ribeiro N, Woodwards R, Thakker N, Sloan P. Fragile histidine triad expression in oral squamous cell carcinoma and precursor lesions. Clin Cancer Res. 2006;12(22):6723–9.
56. Smith J, Rattay T, McConkey C, Helliwell T, Mehanna H. Biomarkers in dysplasia of the oral cavity: a systematic review. Oral Oncol. 2009;45(8):647–53.
57. Bernier J. Head and neck cancer: multimodality management. New York, NY: Springer; 2011.
58. Kadashetti V, Chaudhary M, Patil S, Gawande M, Shivakumar KM, Patil S, Pramod RC. Analysis of various risk factors affecting potentially malignant disorders and oral cancer patients of Central India. J Cancer Res Ther. 2015;11(2):280–6.
59. Dionne KR, Warnakulasuriya S, Zain RB, Cheong SC. Potentially malignant disorders of the oral cavity: current practice and future directions in the clinic and laboratory. Int J Cancer. 2015;136(3):503–15.
60. Kumar S, Debnath N, Ismail MB, Kumar A, Kumar A, Badiyani BK, Dubey PK, Sukhtankar LV. Prevalence and risk factors for oral potentially malignant disorders in Indian population. Adv Prev Med. 2015;2015:208519.
61. Gupta B, Johnson NW. Systematic review and meta-analysis of association of smokeless tobacco and of betel quid without tobacco with incidence of oral cancer in South Asia and the Pacific. PLoS One. 2014;9(11):e113385.
62. Franco T, Trapasso S, Puzzo L, Allegra E. Electronic cigarette: role in the primary prevention of oral cavity cancer. Clin Med Insights Ear Nose Throat. 2016;9:7–12.
63. Holliday R, Kist R, Bauld L. E-cigarette vapour is not inert and exposure can lead to cell damage. Evid Based Dent. 2016;17(1):2–3.
64. Yu V, Rahimy M, Korrapati A, Xuan Y, Zou AE, Krishnan AR, Tsui T, Aguilera JA, Advani S, Crotty Alexander LE, Brumund KT, Wang-Rodriguez J, Ongkeko WM. Electronic cigarettes induce DNA strand breaks and cell death independently of nicotine in cell lines. Oral Oncol. 2016;52:58–65.

65. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? Cancer. 2007;110(7):1429–35.
66. Li L, Psoter WJ, Buxo CJ, Elias A, Cuadrado L, Morse DE. Smoking and drinking in relation to oral potentially malignant disorders in Puerto Rico: a case-control study. BMC Cancer. 2011;11:324.
67. Khan Z, Khan S, Christianson L, Rehman S, Ekwunife O, Samkange-Zeeb F. Smokeless tobacco and oral potentially malignant disorders in South Asia: a protocol for a systematic review. Syst Rev. 2016;5(1):142.
68. Ram H, Sarkar J, Kumar H, Konwar R, Bhatt ML, Mohammad S. Oral cancer: risk factors and molecular pathogenesis. J Maxillofac Oral Surg. 2011;10(2):132–7.
69. Solomon PR, Selvam GS, Shanmugam G. Polymorphism in ADH and MTHFR genes in oral squamous cell carcinoma of Indians. Oral Dis. 2008;14(7):633–9.
70. Petti S, Scully C. Association between different alcoholic beverages and leukoplakia among non- to moderate-drinking adults: a matched case-control study. Eur J Cancer (Oxford, England: 1990). 2006;42(4):521–7.
71. Amarasinghe HK, Johnson NW, Lalloo R, Kumaraarachchi M, Warnakulasuriya S. Derivation and validation of a risk-factor model for detection of oral potentially malignant disorders in populations with high prevalence. Br J Cancer. 2010;103(3):303–9.
72. McCullough MJ, Farah CS. The role of alcohol in oral carcinogenesis with particular reference to alcohol-containing mouthwashes. Aust Dent J. 2008;53(4):302–5.
73. Currie S, Farah C. Alcohol-containing mouthwash and oral cancer risk: a review of current evidence. OA Alcohol. 2014;2(1):4.
74. Dost F, Le Cao KA, Ford PJ, Farah CS. A retrospective analysis of clinical features of oral malignant and potentially malignant disorders with and without oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(6):725–33.
75. Syrjanen S, Lodi G, von Bultzingslowen I, Aliko A, Arduino P, Campisi G, Challacombe S, Ficarra G, Flaitz C, Zhou HM, Maeda H, Miller C, Jontell M. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis. 2011;17(Suppl 1):58–72.
76. Cox M, Maitland N, Scully C. Human herpes simplex-1 and papillomavirus type 16 homologous DNA sequences in normal, potentially malignant and malignant oral mucosa. Eur J Cancer B Oral Oncol. 1993;29B(3):215–9.
77. Starr JR, Daling JR, Fitzgibbons ED, Madeleine MM, Ashley R, Galloway DA, Schwartz SM. Serologic evidence of herpes simplex virus 1 infection and oropharyngeal cancer risk. Cancer Res. 2001;61(23):8459–64.
78. Reddy SS, Sharma S, Mysorekar V. Expression of Epstein-Barr virus among oral potentially malignant disorders and oral squamous cell carcinomas in the South Indian tobacco-chewing population. J Oral Pathol Med. 2017;46:454.
79. Krogh P, Hald B, Holmstrup P. Possible mycological etiology of oral mucosal cancer: catalytic potential of infecting Candida albicans and other yeasts in production of N-nitrosobenzylmethylamine. Carcinogenesis. 1987;8(10):1543–8.
80. Sankari SL, Gayathri K, Balachander N, Malathi L. Candida in potentially malignant oral disorders. J Pharm Bioallied Sci. 2015;7(Suppl 1):S162–4.
81. Mohd Bakri M, Mohd Hussaini H, Rachel Holmes A, David Cannon R, Mary Rich A. Revisiting the association between candidal infection and carcinoma, particularly oral squamous cell carcinoma. J Oral Microbiol. 2010;2
82. Shridhar K, Aggarwal A, Walia GK, Gulati S, Geetha AV, Prabhakaran D, Dhillon PK, Rajaraman P. Single nucleotide polymorphisms as markers of genetic susceptibility for oral potentially malignant disorders risk: review of evidence to date. Oral Oncol. 2016;61:146–51.
83. Prime SS, Thakker NS, Pring M, Guest PG, Paterson IC. A review of inherited cancer syndromes and their relevance to oral squamous cell carcinoma. Oral Oncol. 2001;37(1):1–16.
84. Bishop AJR, Schiestl RH. Role of homologous recombination in carcinogenesis. Exp Mol Pathol. 2003;74(2):94–105.

85. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet. 2009;76(1):1–18.
86. Schoenfeld JD. Immunity in head and neck cancer. Cancer Immunol Res. 2015;3(1):12–7.
87. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546–58.
88. Kang H, Kiess A, Chung CH. Emerging biomarkers in head and neck cancer in the era of genomics. Nat Rev Clin Oncol. 2015;12(1):11–26.
89. Campbell JD, Mazzilli SA, Reid ME, Dhillon SS, Platero S, Beane J, Spira AE. The case for a pre-cancer genome atlas (PCGA). Cancer Prev Res (Phila). 2016;9(2):119–24.
90. Kensler TW, Spira A, Garber JE, Szabo E, Lee JJ, Dong Z, Dannenberg AJ, Hait WN, Blackburn E, Davidson NE, Foti M, Lippman SM. Transforming cancer prevention through precision medicine and immune-oncology. Cancer Prev Res (Phila). 2016;9(1):2–10.
91. William WN Jr, Papadimitrakopoulou V, Lee JJ, Mao L, Cohen EE, Lin HY, Gillenwater AM, Martin JW, Lingen MW, Boyle JO, Shin DM, Vigneswaran N, Shinn N, Heymach JV, Wistuba II, Tang X, Kim ES, Saintigny P, Blair EA, Meiller T, Gutkind JS, Myers J, El-Naggar A, Lippman SM. Erlotinib and the risk of oral cancer: the erlotinib prevention of oral cancer (EPOC) randomized clinical trial. JAMA Oncol. 2016;2(2):209–16.
92. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J Cell Sci. 2007;120(Pt 19):3327–35.
93. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69:89–95.
94. Souto GR, Caliari MV, Lins CE, de Aguiar MC, de Abreu MH, Mesquita RA. Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. J Oral Pathol Med. 2010;39(8):605–10.
95. Bremmer JF, Brakenhoff RH, Broeckaert MA, Belien JA, Leemans CR, Bloemena E, van der Waal I, Braakhuis BJ. Prognostic value of DNA ploidy status in patients with oral leukoplakia. Oral Oncol. 2011;47(10):956–60.
96. Pierssens DDCG, Borgemeester MC, van der Heijden SJH, Peutz-Kootstra CJ, Ruland AM, Haesevoets AM, Kessler PAWH, Kremer B, Speel E-JM. Chromosome instability in tumor resection margins of primary OSCC is a predictor of local recurrence. Oral Oncol. 2017;66:14–21.
97. Gildener-Leapman N, Ferris RL, Bauman JE. Promising systemic immunotherapies in head and neck squamous cell carcinoma. Oral Oncol. 2013;49(12):1089–96.
98. Tan M, Myers JN, Agrawal N. Oral cavity and oropharyngeal squamous cell carcinoma genomics. Otolaryngol Clin North Am. 2013;46(4):545–66.
99. van der Riet P, Nawroz H, Hruban RH, Corio R, Tokino K, Koch W, Sidransky D. Frequent loss of chromosome 9p21-22 early in head and neck cancer progression. Cancer Res. 1994;54(5):1156–8.
100. Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, Hittelman W, Hong WK. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med. 1996;2(6):682–5.
101. Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, Berean K, Epstein JB, Priddy R, Le ND, Zhang L. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res. 2000;6(2):357–62.
102. Johnson SD, Levingston C, Young MR. Premalignant oral lesion cells elicit increased cytokine production and activation of T-cells. Anticancer Res. 2016;36(7):3261–70.
103. Malumbres M. miRNAs and cancer: an epigenetics view. Mol Aspects Med. 2013;34(4):863–74.
104. Cervigne NK, Reis PP, Machado J, Sadikovic B, Bradley G, Galloni NN, Pintilie M, Jurisica I, Perez-Ordonez B, Gilbert R, Gullane P, Irish J, Kamel-Reid S. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. Hum Mol Genet. 2009;18(24):4818–29.

105. Jung HM, Phillips BL, Patel RS, Cohen DM, Jakymiw A, Kong WW, Cheng JQ, Chan EK. Keratinization-associated miR-7 and miR-21 regulate tumor suppressor reversion-inducing cysteine-rich protein with kazal motifs (RECK) in oral cancer. J Biol Chem. 2012;287(35):29261–72.
106. Harrandah AM, Fitzpatrick SG, Smith MH, Wang D, Cohen DM, Chan EK. MicroRNA-375 as a biomarker for malignant transformation in oral lesions. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(6):743–752.e741.
107. Harris T, Jimenez L, Kawachi N, Fan JB, Chen J, Belbin T, Ramnauth A, Loudig O, Keller CE, Smith R, Prystowsky MB, Schlecht NF, Segall JE, Childs G. Low-level expression of miR-375 correlates with poor outcome and metastasis while altering the invasive properties of head and neck squamous cell carcinomas. Am J Pathol. 2012;180(3):917–28.
108. Hung KF, Liu CJ, Chiu PC, Lin JS, Chang KW, Shih WY, Kao SY, Tu HF. MicroRNA-31 upregulation predicts increased risk of progression of oral potentially malignant disorder. Oral Oncol. 2016;53:42–7.
109. Ebert MS, Sharp PA. Emerging roles for natural microRNA sponges. Curr Biol. 2010;20(19):R858–61.
110. Clark MB, Mattick JS. Long noncoding RNAs in cell biology. Semin Cell Dev Biol. 2011;22(4):366–76.
111. Zou AE, Ku J, Honda TK, Yu V, Kuo SZ, Zheng H, Xuan Y, Saad MA, Hinton A, Brumund KT, Lin JH, Wang-Rodriguez J, Ongkeko WM. Transcriptome sequencing uncovers novel long noncoding and small nucleolar RNAs dysregulated in head and neck squamous cell carcinoma. RNA. 2015;21(6):1122–34.
112. Nohata N, Abba MC, Gutkind JS. Unraveling the oral cancer lncRNAome: identification of novel lncRNAs associated with malignant progression and HPV infection. Oral Oncol. 2016;59:58–66.
113. Junttila MR, Evan GI. p53--a Jack of all trades but master of none. Nat Rev Cancer. 2009;9(11):821–9.
114. Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM. The cancer genome atlas pan-cancer analysis project. Nat Genet. 2013;45(10):1113–20.
115. Dassonville O, Formento JL, Francoual M, Ramaioli A, Santini J, Schneider M, Demard F, Milano G. Expression of epidermal growth factor receptor and survival in upper aerodigestive tract cancer. J Clin Oncol. 1993;11(10):1873–8.
116. Bates T, Kennedy M, Diajil A, Goodson M, Thomson P, Doran E, Farrimond H, Thavaraj S, Sloan P, Kist R, Robinson M. Changes in epidermal growth factor receptor gene copy number during oral carcinogenesis. Cancer Epidemiol Biomarkers Prev. 2016;25(6):927–35.
117. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer. 2010;10(2):116–29.
118. Gudigenavar A, Adhyaru P, Naik V. Tumor suppressor genes in oral cancer. Clin Cancer Investig J. 2015;4(6):697–702.
119. Koole K, van Kempen PM, van Bockel LW, Smets T, van der Klooster Z, Dutman AC, Peeters T, Koole R, van Diest P, van Es RJ, Willems SM. FGFR4 is a potential predictive biomarker in oral and oropharyngeal squamous cell carcinoma. Pathobiology. 2015;82(6):280–9.
120. Koole K, van Kempen PM, Swartz JE, Peeters T, van Diest PJ, Koole R, van Es RJ, Willems SM. Fibroblast growth factor receptor 3 protein is overexpressed in oral and oropharyngeal squamous cell carcinoma. Cancer Med. 2016;5(2):275–84.
121. Knowles LM, Stabile LP, Egloff AM, Rothstein ME, Thomas SM, Gubish CT, Lerner EC, Seethala RR, Suzuki S, Quesnelle KM, Morgan S, Ferris RL, Grandis JR, Siegfried JM. HGF and c-Met participate in paracrine tumorigenic pathways in head and neck squamous cell cancer. Clin Cancer Res. 2009;15(11):3740–50.
122. Rousseau A, Lim MS, Lin Z, Jordan RC. Frequent cyclin D1 gene amplification and protein overexpression in oral epithelial dysplasias. Oral Oncol. 2001;37(3):268–75.

123. Mishra R, Das BR. Cyclin D1 expression and its possible regulation in chewing tobacco mediated oral squamous cell carcinoma progression. Arch Oral Biol. 2009;54(10): 917–23.
124. Nasser W, Flechtenmacher C, Holzinger D, Hofele C, Bosch FX. Aberrant expression of p53, p16INK4a and Ki-67 as basic biomarker for malignant progression of oral leukoplakias. J Oral Pathol Med. 2011;40(8):629–35.
125. Izumchenko E, Sun K, Jones S, Brait M, Agrawal N, Koch W, McCord CL, Riley DR, Angiuoli SV, Velculescu VE, Jiang WW, Sidransky D. Notch1 mutations are drivers of oral tumorigenesis. Cancer Prev Res (Phila). 2015;8(4):277–86.
126. Vinay DS, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, Lichtor T, Decker WK, Whelan RL, Kumara HM, Signori E, Honoki K, Georgakilas AG, Amin A, Helferich WG, Boosani CS, Guha G, Ciriolo MR, Chen S, Mohammed SI, Azmi AS, Keith WN, Bilsland A, Bhakta D, Halicka D, Fujii H, Aquilano K, Ashraf SS, Nowsheen S, Yang X, Choi BK, Kwon BS. Immune evasion in cancer: mechanistic basis and therapeutic strategies. Semin Cancer Biol. 2015;35(Suppl):S185–98.
127. Dalley AJ, Abdul Majeed AA, Pitty LP, Major AG, Farah CS. LGR5 expression in oral epithelial dysplasia and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119(4):436–440.e431.
128. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. Cancer. 2010;116(9):2166–73.
129. Pannone G, Rodolico V, Santoro A, Lo Muzio L, Franco R, Botti G, Aquino G, Pedicillo MC, Cagiano S, Campisi G, Rubini C, Papagerakis S, De Rosa G, Tornesello ML, Buonaguro FM, Staibano S, Bufo P. Evaluation of a combined triple method to detect causative HPV in oral and oropharyngeal squamous cell carcinomas: p16 Immunohistochemistry, Consensus PCR HPV-DNA, and In Situ Hybridization. Infect Agent Cancer. 2012;7:4.
130. Fregonesi PA, Teresa DB, Duarte RA, Neto CB, de Oliveira MR, Soares CP. p16(INK4A) immunohistochemical overexpression in premalignant and malignant oral lesions infected with human papillomavirus. J Histochem Cytochem. 2003;51(10):1291–7.
131. Seoane JM, Varela-Centelles PI, Ramirez JR, Cameselle-Teijeiro J, Romero MA, Aguirre JM. Heat shock proteins (HSP70 and HSP27) as markers of epithelial dysplasia in oral leukoplakia. Am J Dermatopathol. 2006;28(5):417–22.
132. Camisasca DR, Honorato J, Bernardo V, da Silva LE, da Fonseca EC, de Faria PA, Dias FL, Lourenco Sde Q. Expression of Bcl-2 family proteins and associated clinicopathologic factors predict survival outcome in patients with oral squamous cell carcinoma. Oral Oncol. 2009;45(3):225–33.
133. Zhang M, Li J, Wang L, Tian Z, Zhang P, Xu Q, Zhang C, Wei F, Chen W. Prognostic significance of p21, p27 and survivin protein expression in patients with oral squamous cell carcinoma. Oncol Lett. 2013;6(2):381–6.
134. Shay JW, Wright WE. The use of telomerized cells for tissue engineering. Nat Biotechnol. 2000;18(1):22–3. https://doi.org/10.1038/71872.
135. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005;6(8):611–22.
136. Park YJ, Kim EK, Bae JY, Moon S, Kim J. Human telomerase reverse transcriptase (hTERT) promotes cancer invasion by modulating cathepsin D via early growth response (EGR)-1. Cancer Lett. 2016;370(2):222–31.
137. Kim HR, Christensen R, Park NH, Sapp P, Kang MK, Park NH. Elevated expression of hTERT is associated with dysplastic cell transformation during human oral carcinogenesis in situ. Clin Cancer Res. 2001;7(10):3079–86.
138. Dorji T, Monti V, Fellegara G, Gabba S, Grazioli V, Repetti E, Marcialis C, Peluso S, Di Ruzza D, Neri F, Foschini MP. Gain of hTERC: a genetic marker of malignancy in oral potentially malignant lesions. Hum Pathol. 2015;46(9):1275–81.

139. Patel MM, Parekh LJ, Jha FP, Sainger RN, Patel JB, Patel DD, Shah PM, Patel PS. Clinical usefulness of telomerase activation and telomere length in head and neck cancer. Head Neck. 2002;24(12):1060–7.
140. Aida J, Izumo T, Shimomura N, Nakamura K, Ishikawa N, Matsuura M, Poon SS, Fujiwara M, Sawabe M, Arai T, Takubo K. Telomere lengths in the oral epithelia with and without carcinoma. Eur J Cancer. 2010;46(2):430–8.
141. Zhou CX, Gao Y, Johnson NW, Gao J. Immunoexpression of matrix metalloproteinase-2 and matrix metalloproteinase-9 in the metastasis of squamous cell carcinoma of the human tongue. Aust Dent J. 2010;55(4):385–9.
142. Ghallab NA, Shaker OG. Serum and salivary levels of chemerin and MMP-9 in oral squamous cell carcinoma and oral premalignant lesions. Clin Oral Investig. 2017;21:937.
143. Astarita JL, Acton SE, Turley SJ. Podoplanin: emerging functions in development, the immune system, and cancer. Front Immunol. 2012;3:283.
144. Feng JQ, Mi JG, Wu L, Ma LW, Shi LJ, Yang X, Liu W, Zhang CP, Zhou ZT. Expression of podoplanin and ABCG2 in oral erythroplakia correlate with oral cancer development. Oral Oncol. 2012;48(9):848–52.
145. Habiba U, Kitamura T, Yanagawa-Matsuda A, Higashino F, Hida K, Totsuka Y, Shindoh M. HuR and podoplanin expression is associated with a high risk of malignant transformation in patients with oral preneoplastic lesions. Oncol Lett. 2016;12(5):3199–207.
146. Nguyen CT, Okamura T, Morita KI, Yamaguchi S, Harada H, Miki Y, Izumo T, Kayamori K, Yamaguchi A, Sakamoto K. LAMC2 is a predictive marker for the malignant progression of leukoplakia. J Oral Pathol Med. 2017;46:223.
147. Kawasaki G, Kato Y, Mizuno A. Cathepsin expression in oral squamous cell carcinoma: relationship with clinicopathologic factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;93(4):446–54.
148. Strauss DC, Thomas JM. Transmission of donor melanoma by organ transplantation. Lancet Oncol. 2010;11(8):790–6.
149. Costa NL, Valadares MC, Souza PP, Mendonca EF, Oliveira JC, Silva TA, Batista AC. Tumor-associated macrophages and the profile of inflammatory cytokines in oral squamous cell carcinoma. Oral Oncol. 2013;49(3):216–23.
150. Mori K, Haraguchi S, Hiori M, Shimada J, Ohmori Y. Tumor-associated macrophages in oral premalignant lesions coexpress CD163 and STAT1 in a Th1-dominated microenvironment. BMC Cancer. 2015;15:573.
151. Lin L, Wang J, Liu D, Liu S, Xu H, Ji N, Zhou M, Zeng X, Zhang D, Li J, Chen Q. Interleukin-37 expression and its potential role in oral leukoplakia and oral squamous cell carcinoma. Sci Rep. 2016;6:26757.
152. Ohman J, Mowjood R, Larsson L, Kovacs A, Magnusson B, Kjeller G, Jontell M, Hasseus B. Presence of CD3-positive T-cells in oral premalignant leukoplakia indicates prevention of cancer transformation. Anticancer Res. 2015;35(1):311–7.
153. Ohman J, Magnusson B, Telemo E, Jontell M, Hasseus B. Langerhans cells and T cells sense cell dysplasia in oral leukoplakias and oral squamous cell carcinomas--evidence for immunosurveillance. Scand J Immunol. 2012;76(1):39–48.
154. Malaspina TS, Gasparoto TH, Costa MR, de Melo EF Jr, Ikoma MR, Damante JH, Cavassani KA, Garlet GP, da Silva JS, Campanelli AP. Enhanced programmed death 1 (PD-1) and PD-1 ligand (PD-L1) expression in patients with actinic cheilitis and oral squamous cell carcinoma. Cancer Immunol Immunother. 2011;60(7):965–74.
155. Troeltzsch M, Woodlock T, Pianka A, Otto S, Troeltzsch M, Ehrenfeld M, Knosel T. Is there evidence for the presence and relevance of the PD-1/PD-L1 pathway in oral squamous cell carcinoma? Hints from an immunohistochemical study. J Oral Maxillofac Surg. 2017;75:969.
156. Peltomaki P. Deficient DNA mismatch repair: a common etiologic factor for colon cancer. Hum Mol Genet. 2001;10(7):735–40.
157. Pimenta FJ, Pinheiro MD, Gomez RS. Expression of hMSH2 protein of the human DNA mismatch repair system in oral lichen planus. Int J Med Sci. 2004;1(3):146–51.

158. Jessri M, Dalley AJ, Farah CS. MutSalpha and MutLalpha immunoexpression analysis in diagnostic grading of oral epithelial dysplasia and squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119(1):74–82.
159. Jessri M, Dalley AJ, Farah CS. hMSH6: a potential diagnostic marker for oral carcinoma in situ. J Clin Pathol. 2015;68(1):86–90.
160. Harris L, Davenport J, Neale G, Goorha R. The mitotic checkpoint gene BubR1 has two distinct functions in mitosis. Exp Cell Res. 2005;308(1):85–100.
161. Rizzardi C, Torelli L, Barresi E, Schneider M, Canzonieri V, Biasotto M, Di Lenarda R, Melato M. BUBR1 expression in oral squamous cell carcinoma and its relationship to tumor stage and survival. Head Neck. 2011;33(5):727–33.
162. Chou CK, Wu CY, Chen JY, Ng MC, Wang HM, Chen JH, Yuan SS, Tsai EM, Chang JG, Chiu CC. BubR1 acts as a promoter in cellular motility of human oral squamous cancer cells through regulating MMP-2 and MMP-9. Int J Mol Sci. 2015;16(7):15104–17.
163. Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, Zhao M, Ortega Alves MV, Chang K, Drummond J, Cortez E, Xie TX, Zhang D, Chung W, Issa JP, Zweidler-McKay PA, Wu X, El-Naggar AK, Weinstein JN, Wang J, Muzny DM, Gibbs RA, Wheeler DA, Myers JN, Frederick MJ. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. Cancer Discov. 2013;3(7):770–81.
164. Zanaruddin SN, Yee PS, Hor SY, Kong YH, Ghani WM, Mustafa WM, Zain RB, Prime SS, Rahman ZA, Cheong SC. Common oncogenic mutations are infrequent in oral squamous cell carcinoma of Asian origin. PLoS One. 2013;8(11):e80229.

Chapter 4
Oral Leukoplakia

Rajiv S. Desai, Ravikant S. Ganga, and Raghav C. Dwivedi

Introduction

Oral leukoplakia (New Latin *leuko*, white, + classical Greek *plax*, flat object) is said to represent the most common premalignant oral mucosal lesion with an estimated global prevalence rate of 2.6%. Historically, practitioners have used many synonyms such as leucoma, smokers' patch, leukokeratosis and ichthyosis for this lesion. In 1851, Sir James Paget documented a connection between a white keratotic oral lesion and lingual carcinoma; however, the term "leukoplakia" has given rise to considerable misunderstanding since it was first defined more than 140 years ago by the dermatologist Ernst Schwimmer in 1877 [1].

Over the ensuing decades, various studies were carried out on oral leukoplakia thus definitively establishing it as a precancerous lesion. However, the frequency of malignant transformation is reported to be widely varied with some studies indicating rates of malignant transformation while some reports being limited to the development of dysplasia within leukoplakia [2]. Irrespective of whether some leukoplakias eventually progress to cancer or not, most of these lesions arise secondary to the use of tobacco, alcohol and betel quid, while the term "idiopathic leukoplakias" is reserved for a few of these lesions that are thought to be genetically destined [3].

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Definition and Terminology

The definition and terminology of oral leukoplakia have been the subject of discussion for many decades. In 1978, the World Health Organization (WHO) first defined oral leukoplakia as "a white patch or plaque that cannot be characterized clinically or pathologically as any other disease". However, this definition emphasized that the term leukoplakia be used in a descriptive clinical sense only with no histologic association. In 1984, the First International Conference on oral leukoplakia in Malmo, Sweden, proposed that only white lesions associated with tobacco consumption be referred to as leukoplakia and defined it as "a whitish patch or plaque that cannot be characterized, clinically or pathologically, as any other disease and it is not associated with any physical or clinical causative agent except the use of tobacco" [4]. Axell et al. in 1994 proposed a new definition of leukoplakia, which stated that "oral leukoplakia is a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion; some oral leukoplakias will transform into cancer". Shortly after that, the definition was modified as "a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion" [5]. This was followed in 2005, by the WHO definition of leukoplakia, "a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer". Finally, in 2007, the World Health Organization Collaborating Centre for Oral Cancer and Precancer reached a consensus at a workshop in the United Kingdom regarding the use of the term "potentially malignant disorders". It was agreed to limit the use of the term to refer to a precancer only since not all disorders designated under the term "potentially malignant disorders" could transform into a malignancy. It was also recommended that having excluded other known diseases or disorders that bring no increased risk for malignancy, the word "leukoplakia" should be used to distinguish "white plaques of debatable risks". Thus, the WHO finally defined this lesion as "a white plaque with a growing debatable oral cancer risk after excluding other known diseases and disorders that do not increase the risk".

However, a debate still exists over the "ideal" definition of leukoplakia that encompasses all the aspects of leukoplakia without any ambiguity. As a result of this debate, "widely accepted" definitions of leukoplakia exist within the scientific community till date. Hence, when a lesion cannot be clearly diagnosed as any other "known disease or disorder" with a white appearance on clinical examination (Table 4.1), only a provisional diagnosis is recorded, and the diagnosis of leukoplakia is one by exclusion of these "known diseases or disorders". While an experienced clinician may find it relatively easy to distinguish many, if not most, of these conditions from leukoplakia on the basis of history and clinical appearance, this may not be the case for the less experienced clinician. This has led professionals to recommend the development of a framework for diagnosing oral leukoplakia that prevents the misclassification of other oral white disorders as leukoplakia. The definition and terminology of a few of the "known" conditions listed in Table 4.1 deserve further attention.

Table 4.1 The most common definable white or predominantly white lesions of the oral mucosa and their main diagnostic criteria

Lesion	Main diagnostic criteria
Candidiasis	Pseudomembranes, often symmetrical pattern
Discoid lupus erythematosus	History of skin lesion; clinical appearance (incl. bilateral pattern); histopathology
Frictional lesion	Presence of mechanical irritation
Hairy leukoplakia	Clinical aspect (incl. bilateral localisation on the tongue); histopathology (incl. EBV)
Leukoedema	Symmetrical pattern
Lichen planus	Symmetrical pattern; histopathology
Morsicatio	History of habitual biting or chewing; clinical aspect
Papilloma and allied lesions	Clinical aspect and histopathology
Tobacco-induced lesions (smoker's palate, palatal lesions in reverse smoking, snuff dippers' lesion)	Clinical aspect; history of smoking; history of reverse smoking; site where snuff is placed
White sponge nevus	Family history; clinical aspect (often symmetrical pattern)

Alveolar Ridge Keratosis and Frictional Keratosis

Alveolar ridge keratosis apparently results from frictional trauma to the maxillary and mandibular alveolar ridges that is chronic in nature and has been discussed by a few authors [6, 7]. Almost all lesions are known to exhibit hyperkeratosis without the presence of epithelial dysplasia histopathologically. Frictional keratosis of the facial (buccal) attached gingiva, another reversible white lesion which is caused by mechanical irritation of the mucosa, is known to show some overlapping features with this condition. However, a definitive diagnosis in case of a lesion appearing due to friction or mechanical irritation can only be made in retrospect. This diagnostic approach involves the elimination of the possible cause of mechanical irritation following which the lesion may disappear.

Hyperplastic Candidiasis

Clinically, a distinction between these lesions particularly those located at the commissures of the lips, the hard palate and the dorsal surface of the tongue, can be made by treatment with antifungal medication for a period of 4–8 weeks. Lesions that disappear following antifungal treatment need not be referred to as leukoplakias any longer. However, a diagnosis of *Candida*-associated leukoplakia should be considered in case of persistent lesions.

Oral Hairy Leukoplakia (Greenspan Lesion)

In 1984, Greenspan and her colleagues introduced the term oral hairy leukoplakia presenting as an alteration along the lateral border of the tongue in male homosexuals and on the buccal or labial mucosa of immunocompromised individuals. Early lesions tend to be smooth on appearance eventually becoming heavily folded and vertically corrugated over time. Histologically, no evidence of dysplasia is noted in the deeper layers of the epithelium. There is also no reported association with malignant transformation [2]. Since it is a definable lesion even though the clinical aspect is not always "hairy", the term hairy leukoplakia is considered to be a misnomer. Additionally, it is not considered to be a potentially malignant disorder since biopsy specimens reveal the presence of EBV DNA in the koilocytic epithelial cells on immunohistochemical analysis [8]. As a result, it has been suggested that the term "Greenspan lesion" be used instead of hairy leukoplakia, which should be abandoned [9].

Smokers' Lesions

Smoker's palate (nicotine stomatitis), palatal keratosis in reverse smokers and snuff dippers' lesions are usually listed as "definable lesions" since they are tobacco-induced and are not defined as leukoplakia [4, 10, 11]. Nicotine stomatitis is a generalized white palatal alteration that seems to be a hyperkeratotic response to the heat generated by tobacco smoking rather than a response to the carcinogens within the smoke. As a result, it is not considered to be a precancerous lesion. Its malignant transformation potential is low enough to be about the same as that of normal palatal mucosa. However, these lesions are known to regress within an arbitrary period of no more than 4–8 weeks if patients discontinue their habits. In such cases, the provisional clinical diagnosis should, in retrospect, be changed into "smokers' lesion". In case the lesion persists due to the unwillingness of the patient to give up the tobacco habit, the term "tobacco-associated leukoplakia" can be applied [8, 12].

Epidemiology

Incidence and Prevalence

Leukoplakia represents 85% of all the oral potentially malignant disorders thus being the most common of such lesions. The prevalence of oral leukoplakia exhibits a significant geographical variation in South East Asia which appears to be influenced by various aetiological factors. In a 10-year prospective study in India in large random samples, carried out in several geographic areas with various kinds of tobacco usage, the annual age-adjusted incidence rates of leukoplakia per 1000 population per year varied from 1.1 to 2.4 among men and from 0.2 to 1.3 among

women; the prevalence varied from 0.2% to 4.9% [13]. The estimate for prevalence in global terms is 2.6%, and prevalence in developed countries is around 3% [1, 14].

Age and Gender

The onset of leukoplakia usually takes place after the age of 30 years, resulting in a peak incidence above the age of 40 years. Globally, there is a strong male predilection (70%), except in regional populations in which women use tobacco products more than men [15].

Aetiology

The aetiology of oral leukoplakia is considered to be multifactorial; however, smoking remains the predominant causative factor globally. The frequency of occurrence of the lesion is hence found to be more in smokers than in non-smokers. However, in South East Asia, an increased prevalence of these lesions is attributed to the betel nut chewing habit. The role of alcohol as an independent aetiological factor in the development of oral leukoplakia is still questionable. Except in the case of smoking, establishing an aetiological factor for a whitish plaque can help rule out the diagnosis of leukoplakia. Other infections that have been implicated as cofactors that may affect the prognosis of established leukoplakia include *Candida*, human papillomavirus (HPV) and, more recently, Epstein-Barr virus (EBV). The term "idiopathic leukoplakia" is used to describe those lesions that develop in the absence of identifiable risk factors. The development of such lesions is generally considered to have a genetic origin [16, 17]. Nicotine Stomatitis, a hyperkeratotic response to the heat generated by tobacco smoking rather than a response to the carcinogens within the smoke should be differentiated from Oral leukoplakia in a smoker since it is not considered to be a pre-cancer. Its malignant transformation potential is as low as that of normal palatal mucosa [18, 19].

Leukoplakia and Candida

There is still some uncertainty regarding the role of *Candida albicans* in the aetiology and the malignant transformation of leukoplakia. About 10% of oral leukoplakias satisfy the clinical and histological criteria for chronic hyperplastic candidiasis (candidal leukoplakia) which is also four to five times more likely to develop epithelial dysplasia than in leukoplakia. The role of *Candida* species in the aetiology or progression of leukoplakias is debatable. The evidence justifying an aetiological role of candida in malignant transformation of leukoplakia is varied and includes the catalytic transformation, in vitro of the carcinogenic nitrosamine, *N*-nitrosobenzyl-methyl-lamine, by strains of *C. albicans* demonstrated to be selectively associated with leukoplakia [20, 21].

Leukoplakia and Human Papillomavirus

The possibility of human papillomavirus (HPV) as an aetiological agent and its role in the malignant transformation of oral premalignant lesions have been studied extensively. However, its role as a definite causative agent in the development of oral leukoplakia still remains questionable. The most studied HPV types included types 6, 11, 16, 18, 31, 33 and 35, which can also be found in normal oral epithelium; thus their presence can be considered coincidental or simply represents a super infection [22–24].

Leukoplakia and Epstein-Barr Virus

The role of Epstein-Barr virus (EBV) as an aetiological agent has been implicated in various cancers including oral squamous cell carcinoma. Bagán et al. [25], in their study, observed no association between proliferative verrucous leukoplakia and EBV. No conclusive evidence regarding the role of EBV in the development of oral leukoplakia has still been established [25].

A similar paucity of evidence exists regarding the possible role of genetic factors in the development of oral leukoplakia.

Pathogenesis

Abnormal epithelial differentiation, increased surface keratinization and alterations in the epithelial thickness in the form of atrophy or acanthosis (thickened prickle cell layer) represent the primary pathological changes in leukoplakia. Various molecular mechanisms have been implicated in the development of oral leukoplakia and its malignant transformation. These include loss of differentiation-related keratins and carbohydrate antigens and alterations in oncogene/tumour suppression gene expression, oxidative stress and nitrative DNA damage via reactive nitrogen products such as inducible nitric oxide synthase and inflammation-related mechanisms. However, their ability to serve as targets for diagnostic and therapeutic biomarkers remains limited and open to future research [26].

Clinical Features

Leukoplakia can affect any site in the oral cavity either as localized, diffuse or multiple lesions. Its distribution is related to gender and tobacco habits showing worldwide differences. In general, two clinical variants are recognized, the

homogeneous and the non-homogeneous types. The non-homogeneous type includes (1) speckled, (2) nodular/granular and (3) verrucous/verruciform leukoplakia [27].

Homogeneous Leukoplakia

It appears typically uniformly white, relatively flat and superficial with clear demarcated margins (Fig. 4.1) which is free from interspersed areas of fissuring and erythema and without a nodular, verrucous or otherwise irregular surface (Fig. 4.2).

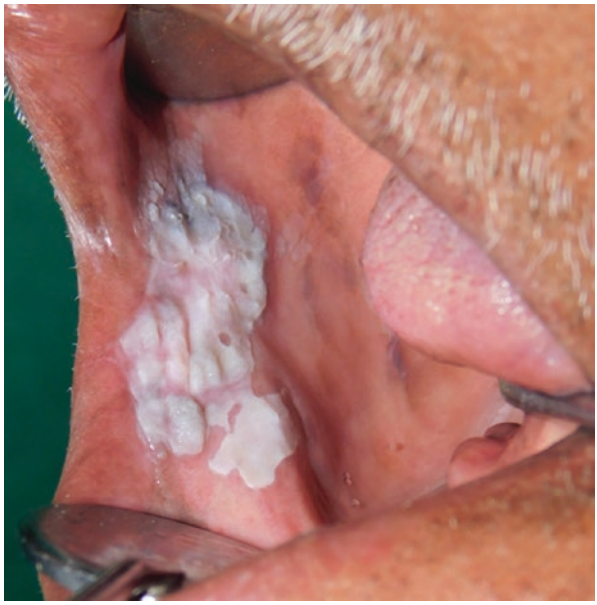

Fig. 4.1 Homogenous leukoplakia affecting the right buccal mucosa

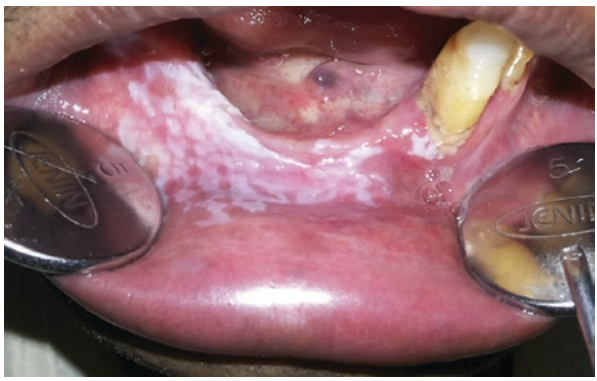

Fig. 4.2 Homogenous leukoplakia in a partially edentulous patient affecting the alveolar ridge and involving the labial vestibule in the anterior mandibular region

Non-homogeneous Leukoplakia

Non-homogenous leukoplakias tend to be less well demarcated and represent a higher-risk lesion as compared to homogenous leukoplakia. Speckled areas or islands of red patches within the oral leukoplakia warrant a revision of the diagnosis to a speckled leukoplakia (Fig. 4.3). Verrucous leukoplakia has an elevated, proliferative or corrugated surface appearance which is quite often misdiagnosed by clinicians as verrucous carcinoma as there are actually no strict criteria to make a distinction between these entities. The nodular type has small polypoid outgrowths with rounded predominantly white excrescences [28].

Erythroplakia of the oral mucosa is defined as "a fiery red patch which cannot be characterised clinically or pathologically as any other definable condition". Even though it is a relatively rare lesion, its diagnosis is considered to be clinically significant since it has the highest rate of malignant transformation (14–50%) of all the oral potentially malignant disorders.

Proliferative Verrucous Leukoplakia (PVL)

It is a rare, recalcitrant, often widespread and multifocal high-risk form of leukoplakia, first described by Hansen et al. in 1985. These lesions start as a simple hyperkeratosis and then spread to become multifocal with a high rate of recurrence [29, 30]. It has a strong predilection for elderly females (1:4, male-to-female ratio) exhibiting a minimal association with tobacco use. The lesions manifest as

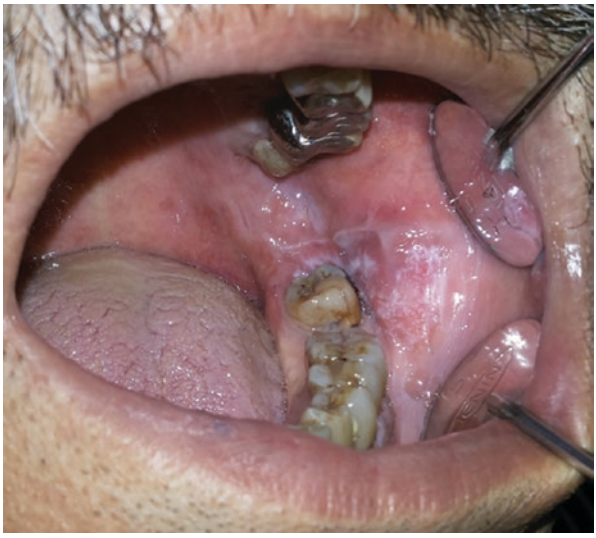

Fig. 4.3 Speckled leukoplakia involving the left buccal mucosa

exophytic, wart-like lesions that enlarge and spread. Major and minor criteria for the diagnosis of PVL had been suggested by Cerero-Lapiedra et al. [31] which have been recently modified to four diagnostic criteria [31]. These include the presence of verrucous or wart-like leukoplakia involving two or more subsites, when added, all the involved sites the minimum size of the lesions should be at least 3 cm; at least 5 years of disease evolution and the availability of at least one biopsy ruling out a verrucous carcinoma or squamous cell carcinoma [32]. Due to its high rate of malignant transformation, especially in lesions with multicentric foci, PVL is considered to be a forerunner of verrucous carcinoma [33].

Histopathological Features

It is worth mentioning that leukoplakia is a clinical term and denotes a negative diagnosis based on exclusion criteria. The term leukoplakia does not carry histological implications. Following a biopsy, the histological diagnosis should replace the term leukoplakia. However, it is recommended that a statement on the presence or absence of epithelial dysplasia with an assessment of its severity be included in the histological report of such lesions.

Epithelial hyperplasia and surface hyperkeratosis are the hallmark histopathological features of leukoplakia. Microscopically, leukoplakia is characterized by a thickened keratin layer of the surface epithelium (hyperkeratosis), with or without a thickened spinous layer (acanthosis). Some leukoplakias demonstrate surface hyperkeratosis but show atrophy or thinning of the underlying epithelium. Frequently, variable numbers of chronic inflammatory cells are notes within the subjacent connective tissue. The keratin layer may consist of parakeratin (hyperparakeratosis), orthokeratin (hyperorthokeratosis) or a combination of both. Verrucous leukoplakia has papillary or pointed surface projections, varying keratin thickness and broad, blunted rete ridges. It may be difficult to differentiate it from early verrucous carcinoma.

Superficial layers of the epithelium may show the formation of microabscesses in the presence of *C. Albicans*, and an inflammatory cell infiltrate is commonly seen. Epithelial dysplasia, if present, may range from mild to severe; however, carcinoma in situ and even squamous cell carcinoma are encountered histologically in some instances. When the dysplastic epithelium shows features that, to some extent, resemble those of lichen planus, the term "lichenoid dysplasia" is sometimes used [34, 35]. Long-time follow-up should be considered in case of exophytic, verrucous or papillomatous lesions as these are known to progress to squamous cell carcinoma in time, in spite of the absence of epithelial dysplasia.

The various cellular as well as architectural changes that may occur in epithelial dysplasia are listed in Table 4.2. Based on the histopathologist's interpretation of the presence of these dysplastic features, epithelial dysplasia is usually divided into three categories: mild, moderate and severe. Due to a strong interobserver discrepancy between pathologists in the evaluation of the presence and the degree of epithelial

Table 4.2 Cellular and architectural changes in epithelial dysplasia

Cellular changes	Architectural changes
Abnormal variation in nuclear size (anisonucleosis)	Loss of polarity
Abnormal variation in cell size (anisocytosis)	Disordered maturation from basal to squamous cells
Increased nuclear/cytoplasmic ratio	Increased cellular density
Enlarged nuclei and cells	Basal cell hyperplasia
Hyperchromatic nuclei	Premature keratosis and keratin pearls deep within the epithelium
Increased mitotic figures	Bulbous drop-shaped rete pegs
Abnormal mitotic figures (abnormal in shape and location)	Secondary extensions on rete tips
Nuclear and cellular pleomorphism	
Increased number and size of nucleoli	

dysplasia, it is difficult to establish a strong correlation between the degree of dysplasia and the rate of transformation of leukoplakia [36, 37]. Since leukoplakia without dysplasia demonstrates a 15% rate of malignant transformation, the absence of histological dysplasia does not guarantee its benign behaviour. Thus it can be concluded that even though histological dysplasia is considered to be an important parameter, malignant transformation of leukoplakia can occur irrespective of its presence or absence [38–40].

Kujan et al. [41] proposed a new classification based on the morphological criteria used by WHO 2005, into high risk and low risk on scoring the features [41]. In the year 2006, the binary system was proposed for grading epithelial dysplasia in oral leukoplakia with the aim of reducing subjectivity and increasing the possibility of conformity between histological interpretations of different pathologists. They divided oral epithelial dysplasia into:

1. High-risk lesions: Presence of five cytological changes and four architectural changes. They have a potential for malignant transformation.
2. Low-risk lesions: It does not show a potential for malignant transformation. It exhibits less than four cytological and architectural changes [28, 42]. Thus, the final diagnosis of the white lesion of the oral mucosa can only be made through a clinicopathological correlation.

Classification and Staging System

In order to promote uniform reporting of various aspects of leukoplakia, the OLEP staging system was proposed by Van der Waal et al. [43] in which the size of the lesion and the presence or absence of dysplasia were taken into consideration [43]. Altogether four stages were recognized:

- L1—Size of leukoplakia ˂2 cm
- L2—Size of leukoplakia 2–4 cm

- L3—Size of leukoplakia ˃4 cm
- Lx—Size not specified
- P—Pathology
- P0—No epithelial dysplasia
- P1—Distinct epithelial dysplasia
- Px—Dysplasia not specified in the pathology report

OLEP Staging System

- Stage I—L1P0
- Stage II—L2P0
- Stage III—L3P0 or L1L2P1
- Stage IV—L3P1

The advantage of this system being that it can easily be adjusted by replacing the histopathological criteria of epithelial dysplasia by a clinical subdivision in homogeneous and non-homogeneous leukoplakia for cases in which no biopsy is available.

Malignant Transformation

No definite morphological, immunological or histochemical criteria have been demonstrated to identify the particular types of leukoplakia that will eventually undergo a malignant transformation. Malignant transformation of oral leukoplakia varies from 0.13% to 34% in different populations and geographic areas. The precise time for the development of a malignant transformation of a leukoplakic patch is not known; however, patients with oral leukoplakia carry a fivefold higher risk of developing oral cancer than controls. Dysplastic lesions are about 15 times more likely to undergo a malignant transformation than non-dysplastic ones [3].

Lesion Distribution

Focal leukoplakia carries a good long-term prognosis as compared to disseminated forms, which affect several sites of the oral mucosa and have a poor prognosis [44].

Lesion Site

The floor of the mouth and the ventrolateral region of the tongue are more exposed to carcinogens in salivary secretions, and the epithelium is more permeable in this area.

Leukoplakias located on the floor of the mouth and in the ventrolateral region of the tongue are associated with a greater risk of malignant transformation (43%) as they are more exposed to carcinogens in salivary secretions and the epithelium is more permeable in this area [45].

Other Characteristics

While evaluating poor prognosis of oral leukoplakias, other characteristics like lesions larger than 20 mm, rapid growth, a previous history of oral squamous cell carcinoma and regular consumption of alcohol or tobacco should also be taken into consideration [46].

Although a strong association is present between human papillomavirus (HPV) and oropharyngeal squamous cell carcinoma (OPSCC), there is little evidence to support a causal link between oral leukoplakia and HPV [47].

Biomarkers for oral carcinogenesis such as markers of proliferation (Ki-67), component of cell cycle control such as tumour suppressor proteins p53, the retinoblastoma protein (pRb) and cyclin D1 are of little help in predicting malignant transformation of leukoplakia. However, in non-dysplastic leukoplakia, a combined alteration of p53/Ki67/p16INK4a was proven to be a risk of progression [48].

Diagnostic Procedures

Elimination of Possible Causes

Before taking a biopsy, elimination of possible causative factors followed by an observation period 2–4 weeks for a possible regression or disappearance of a white lesion is advisable.

Biopsy

Biopsy is the gold standard to rule out malignancy in leukoplakia. In homogenous leukoplakia, a single biopsy or a conservative excisional biopsy is indicated. If treatment consists of CO_2 laser evaporation, it is mandatory to have a biopsy taken prior to such treatment. In non-homogeneous leukoplakias, biopsy should be taken at the sites of redness or induration.

It is advisable to take deeper biopsies preferably from the margins of exophytic, verrucous or papillary lesions to rule out not only the presence of epithelial dysplasia but also the invasion [27].

Adjunctive Techniques

In recent years, attempts have been made to develop new techniques to aid in the identification and diagnosis of premalignant and malignant oral lesions. However, at the present time, careful clinical evaluation with directed conventional biopsy is the best and most accurate means of assessing oral leukoplakic lesions.

Cytology and brush biopsy can be used for mass screening campaigns and involves a non-invasive technique of collecting the basal layer of cells using a brush, thus eliminating the need for a surgical procedure. However, due to many pitfalls and limitations, it should not be considered as a substitute for biopsy when there is concern about malignancy in doubtful lesions [49].

Toluidine blue is a chair-side intravital staining method for nucleic acids and abnormal tissue, used as a guidance for biopsy site selection in suspicious lesions (Fig. 4.4).

Chemiluminescence

The ViziLite system uses reflective tissue fluorescence (chemiluminescence) which is based upon the normal fluorescence of the tissue when exposed to blue-white illumination. It detects a variety of oral mucosa lesions including linea alba, hairy tongue and leukoedema traumatic ulcers [50]. Although oral leukoplakia exhibits a high degree of visibility and sharpness with prominent and distinctive margins of the surrounding mucosa, ViziLite has a limitation in distinguishing benign, inflammatory and potentially malignant disorders as well as a low specificity (28%) in detection of dysplasia and should be used with caution [51].

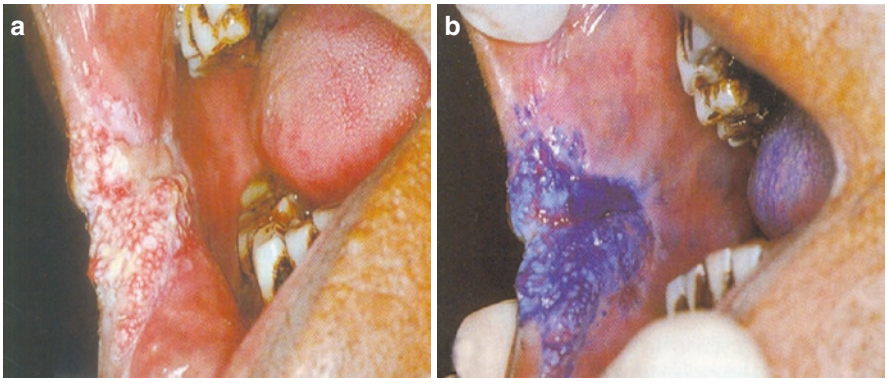

Fig. 4.4 (**a**) Speckled leukoplakia affecting the right commissural mucosa. (**b**) The same lesion after staining with toluidine blue showing a positive staining reaction with dysplastic/inflamed epithelium

Tissue Autofluorescence

Autofluorescence examination of oral tissues is used as an adjunctive tool for early detection and diagnosis of oral cancer. The Visually Enhanced Lesion Scope (VELscope) utilizes direct tissue autofluorescence to enhance oral mucosal abnormalities. A pale green autofluorescence at the excitation wavelengths (375–440 nm) is associated with normal, unaltered mucosa, while dysplastic tissues exhibit loss of tissue autofluorescence and appear darker in colour due to a disruption in the distribution of the fluorochromes.

Oral leukoplakias demonstrate increased autofluorescence when compared to normal mucosa due to an increase in keratinization, which limits the ability of the VELscope to detect malignant change within leukoplakia (Fig. 4.5). Therefore, autofluorescence examination demonstrates a lower specificity for discriminating dysplasias and cancers from benign lesions, and the VELscope cannot provide a definitive diagnosis as to the presence of dysplastic tissue change in oral leukoplakias [52].

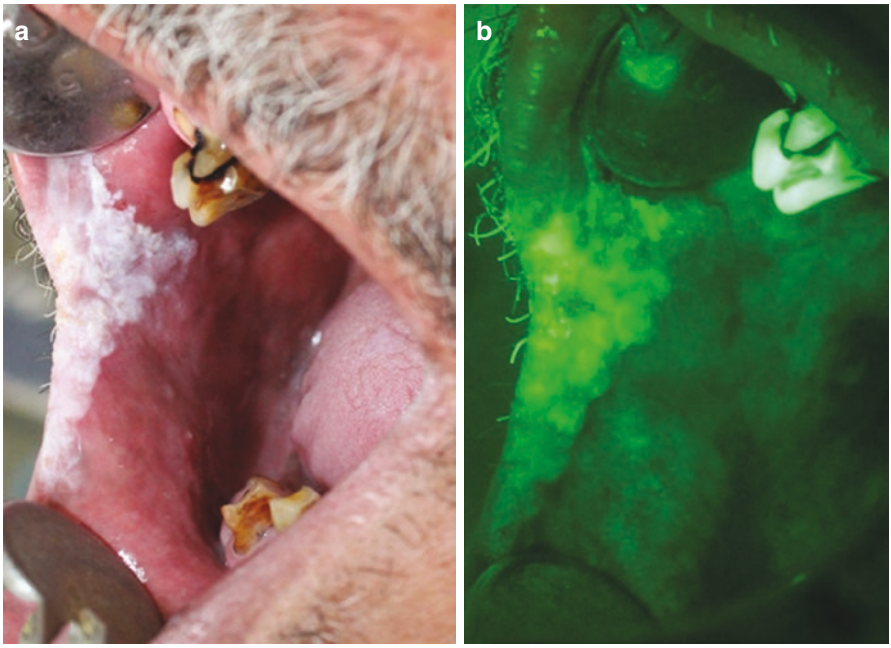

Fig. 4.5 (**a**) Homogenous leukoplakia affecting the right commissural mucosa. (**b**) Autofluorescence examination of the same lesion using VELscope demonstrating an increased tissue autofluorescence when compared to the adjacent normal mucosa

Salivary Biomarkers

Despite extensive research in the field of salivary biomarkers, not a single biomarker has yet been established for routine use in clinical practice for early detection of oral cancer in oral leukoplakia [29].

Management

There is no consensus regarding the most appropriate treatment for oral leukoplakia. Because leukoplakia represents a clinical term only, a biopsy for the assessment of epithelial dysplasia guides the course of treatment. A surgical treatment is recommended in the presence of moderate or severe epithelial dysplasia. However, excision of low-risk oral leukoplakia depends on the location, size and, in the case of smokers, patient's engagement in smoking cessation. Even though topical bleomycin, systemic retinoids and systemic lycopene have been used as nonsurgical therapeutic options to treat oral leukoplakias, their effectiveness is debatable due to inadequate evidence and the lack of long-term studies. Invasive therapeutic modalities include cryosurgery, carbon dioxide laser light and surgical resection [53, 54].

Preventive Treatment

Primary prevention activities like cessation of habits associated with the use of tobacco and alcohol in all its forms. The availability of tobacco and extent of the habit in the population can be reduced by legislative efforts to restrict the production, sale and product usage.

Public information and health education programmes must be conducted to forbid people from adopting any tobacco habit. Maintenance of good oral hygiene and diet fortified with protein, carbohydrates, fat, vitamins and fruits go a long way in prevention of leukoplakia.

Conservative Treatment

Extraction of offending teeth and grinding of dentures are a must to reduce the constant source of irritation to the buccal mucosa and tongue. Chemoprevention also may be useful but remains primarily experimental. The medical treatment uses local and systemic chemopreventive agents such as vitamin A and retinoids, systemic beta carotene, lycopene (a carotenoid), ketorolac (as mouthwash), local

bleomycin and a mixture of tea used both topically and systemically with a reduced benefit [55–57]. Administration of vitamin A and E preparations has convincingly shown to cause regression of leukoplakic lesions. However, after discontinuation of vitamin A, the lesions have recurred. Toxic reactions to systemic retinoids are frequent, however, as is lesion recurrence after the conclusion of therapy. Topical application of bleomycin has been tried for the treatment of oral leukoplakia. It is to be noted that the results of the treatment appear only 3 months after starting therapy with bleomycin. Once the lesions are cured, their recurrence is slower after surgery. However, lifetime follow-up is mandatory every 6 months for patients with oral leukoplakia irrespective of treatment modality. The invariable presence of *Candida albicans* in leukoplakic lesions necessitates the use of concurrent antifungal drugs.

Surgical Treatment

Complete Excision

The surgical treatment can use conventional surgery or laser ablation, electrocauterization or cryosurgery. However, this treatment modality does not lower the risk of subsequent malignant transformation and recurrence. Cryotherapy and electrocautery are not considered to be a first-line therapy of oral leukoplakia as they carry the risk of post-operative scarring and tissue contraction [57].

Complete excision of the leukoplakic patch, followed by histopathologic examination, is the most commonly performed procedure for small lesions. Large lesions can be treated by excision of the leukoplakic patch followed by split skin grafting or pedicled buccal fat pad graft.

Laser Ablation

Laser excision of the lesion by a CO_2 laser and NJYAG laser has revolutionized the management of leukoplakia. Laser excision has its own advantages. It allows precise excision of the patch and minimizes post-operative pain, induction and slough formation. It also aids in rapid epithelization of the raw area.

Follow-Up

All oral leukoplakias should undergo a lifetime follow-up at regular intervals, which may range from 3 months to 6 months and 6–12 months in high-risk and low-risk patients, respectively.

Conclusion

Oral leukoplakia is a clinical diagnosis without histological connotation. Even though it has a well-documented potential risk of malignant transformation into oral squamous cell carcinoma, biomarkers predicting malignant potential are limited in capability. Hence, it can be said that not every whitish patch in the oral cavity is leukoplakia and not every leukoplakia is a precancer. Due to lack of established treatment protocols, current guidelines advocate complete excision of the lesion whenever possible, regular follow-up to detect any mucosal change and a complete cessation of exposure to major risk factors like tobacco and alcohol.

References

1. Petti S. Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncol. 2003;39:770–80.
2. Sciubba JJ. Oral leukoplakia. Crit Rev Oral Biol Med. 1995;6:147–60.
3. Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. J Oral Pathol Med. 2016;45:155–66.
4. Axell T, Holmstrup P, Kramer IRH, Pindborg JJ, Shear M. International seminar on oral leukoplakia and associated lesions related to tobacco habits. Community Dent Oral Epidemiol. 1984;12:145–54.
5. Pindborg JJ, Reichart PA, Smith CJ, Van der Waal I. Histological typing of cancer and precancer of the oral mucosa. World Health Organization International histological classification of tumours. Berlin: Springer; 1997.
6. Chi AC, Lambert PR, Pan Y, Li R, Vo DT, Edwards E, et al. Is alveolar ridge keratosis a true leukoplakia? A clinicopathologic comparison of 2,153 lesions. J Am Dent Assoc. 2007;138:641–51.
7. Natarajan E, Woo SB. Benign alveolar ridge keratosis (oral lichen simplex chronicus): a distinct clinicopathologic entity. J Am Acad Dermatol. 2008;58:151–7.
8. van der Waal I. Oral leukoplakia, the ongoing discussion on definition and terminology. Med Oral Patol Oral Cir Bucal. 2015;20:e685–92.
9. van der Waal I. Greenspan lesion is a better term than “hairy leukoplakia”. J Oral Pathol Med. 1996;25:144.
10. Axell T, Pindborg JJ, Smith CJ, van der Waal I. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppasala, Sweden, May 18-21, 1994. International collaborative group on oral white lesions. J Oral Pathol Med. 1996;25:49–54.
11. Mehta FS, Hammer JE. Tobacco-related oral mucosal lesions and conditions in India, A guide for dental students, dentists, and physicians. Bombay: Basic Dental Research Unit, Tata Institute of Fundamental Research; 1993.
12. Warnakulasuriya S, Dietrich T, Bornstein MM, Casals PE, Preshaw PM, Walter C, et al. Oral health risks of tobacco use and effects of cessation. Int Dent J. 2010;60:7–30.
13. Gupta PC, Mehta FS, Daftary DK. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. Community Dental and Oral Epidemiology. 1980;8:287–333.
14. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45:317–23.

15. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37:1–10.
16. Khan Z, Khan S, Christianson L, Rehman S, Ekwunife O, Samkange-Zeeb F. Smokeless tobacco and oral potentially malignant disorders in South Asia: a systematic review and meta-analysis. Nicotine Tob Res. 2016;5:142.
17. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; present concepts of management. Oral Oncol. 2010;46:423–5.
18. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics 2000. CA Cancer J Clin. 2000;50:7–33.
19. Paricio-Rubio J, Revenga-Arranz J, Ramírez-Gasca T, Boned-Blas P. Leucoqueratosis nicotínica del paladar. Actas Dermosifiliogr. 2002;93:38–41.
20. Jepsen A, Winther JE. Mycotic infection in oral leukoplakia. Acta Ondontol Scand. 1965;23:239–56.
21. Renstrup G. Occurrence of candida in oral leukoplakias. Acta Pathol Microbiol Scand. 1970;78:421–4.
22. Palefsky JM, Silverman S, Abdel-Salaam M, Daniels TE, Greenspan JS. Association between proliferative verrucous leukoplakia and infection with human papillomavirus type 16. J Oral Pathol Med. 1995;24:193–7.
23. Praetorius F. HPV-associated diseases of oral mucosa. Clin Dermatol. 1997;15:299–413.
24. Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a metaanalysis, 1982-1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91:622–35.
25. Bagán JV, Jiménez Y, Murillo J, Poveda R, Díaz JM, Gavaldá C, et al. Epstein-Barr virus in oral proliferative verrucous leukoplakia and squamous cell carcinoma: a preliminary study. Med Oral Patol Oral Cir Bucal. 2008;13:E110–3.
26. Salati NA, Khwaja KJ, Anwer M. Pathogenesis of potentially malignant disorders. Guident. 2012;5:60–2.
27. van der Waal I, Schepman KP, van der Meij EH, Smeele LE. Oral leukoplakia: a clinicopathological review. Oral Oncol. 1997;33:291–301.
28. Warnakulasuriya S, Johnson NW, Van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36:575–80.
29. Hansen LS, Olson JA, Silverman S. Proliferative verrucous leukoplakia. A long-term study of thirty patients. Oral Surg Oral Med Oral Pathol. 1985;60:285–98.
30. Bagan JV, Jiménez-Soriano Y, Diaz-Fernandez JM, et al. Malignant transformation of proliferative verrucous leukoplakia to oral squamous cell carcinoma: a series of 55 cases. Oral Oncol. 2011;47:732–5.
31. Cerero-Lapiedra R, Baladé-Martínez D, Moreno-López LA, Esparza-Gómez G, Bagán JV. Proliferative verrucous leukoplakia: a proposal for diagnostic criteria. Med Oral Patol Oral Cir Bucal. 2010;15:e839–45.
32. Carrard VC, Brouns EREA, van der Waal I. Proliferative verrucous leukoplakia; a critical appraisal of the diagnostic criteria. Med Oral Patol Oral Cir Bucal. 2013;18:e411–3.
33. Cabay RJ, Morton TH, Epstein JB. Proliferative verrucous leukoplakia and its progression to oral carcinoma: a review of the literature. J Oral Pathol Med. 2007;36:255–61.
34. Pindborg JJ, Reibel J, Roed-Petersen B, Mehta FS. Tobacco-induced changes in oral leukoplakic epithelium. Cancer. 1980;45:2330–6.
35. Daniels TE, Hansen LS, Greenspan JS, et al. Histopathology of smokeless tobacco lesions in professional baseball players. Association with different types of tobacco. Oral Surg Oral Med Oral Pathol. 1992;73:720–5.
36. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;79:321–9.
37. Abbey LM, Kaugars GE, Gunsolley JC, et al. Intraexaminer and interexaminer reliability in the diagnosis of oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Endod. 1995;80:188–91.

38. Mincer HH, Coleman SA, Hopkins KP. Observations on the clinical characteristics of oral lesions showing histologic epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1972;33:389–99.
39. Bánóczy J. Follow up studies in oral leukoplakia. J Maxillofac Surg. 1977;5:69–75.
40. Cowan CG, Gregg TA, Napier SS, McKenna SM, Kee F. Potentially malignant oral lesions in northern Ireland: a 20-year population-based perspective of malignant transformation. Oral Dis. 2001;7:18–24.
41. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006;42:987–93.
42. Parlatescu I, Gheorghe C, Coculescu E, Tovaru S. Oral leukoplakia–an update. Maedica. 2014;9:88.
43. van der Waal I, Schepman KP, Van der Meij EH. A modified classification and staging system for oral leukoplakia. Oral Oncol. 2000;36:264–6.
44. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med. 2003;14:47–62.
45. Kramer IR, el-Labban N, Lee KW. The clinical features and risk of malignant transformation in sublingual keratosis. Nr Dent J. 1978;144:171–80.
46. Duncan KO, Geisse JK, Leffel DJ. Epidermal and appendageal tumors. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, editors. Fitzpatrick's dermatology in general medicine. 7th ed. New York, NY: McGraw Hill; 2007. p. 1024–6.
47. Feller L, Lemmer J. Oral leukoplakia as it relates to HPV infection: a review. Int J Dent. 2012;2012:1–7.
48. Nasser W, Flechtenmacher C, Holzinger D, et al. Aberrant expression of p53, p16INK4a and Ki-67 as basic biomarker for malignant progression of oral leukoplakias. J Oral Pathol Med. 2011;40:629–35.
49. Reddy SG, Reddy SG, Kanala S, et al. The sensitivity and specificity of computerized brush biopsy and scalpel biopsy in diagnosing oral premalignant lesions: a comparative study. J Oral Maxillofac Pathol. 2012;16:349–53.
50. Lingen MW, Kalmar JR, Karrison T, et al. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol. 2008;44:10–22.
51. Awan KH, Morgan PR, Warnakulasuriya S. Utility of chemiluminescence (ViziLite™) in the detection of oral potentially malignant disorders and benign keratoses. J Oral Pathol Med. 2011;40:541–4.
52. Ganga RS, Gundre D, Bansal S, Shirsat PM, Prasad P, Desai RS. Evaluation of the diagnostic efficacy and spectrum of autofluorescence of benign, dysplastic and malignant lesions of the oral cavity using VELscope. Oral oncology. 2017;75:67–74.
53. Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A. Interventions for treating oral leukoplakia. Cochrane Database Syst Rev. 2006;(4):CD001829.
54. Brennan M, Migliorati CA, Lockhart PB, Wray D, Al-Hashimi I, Axéll T, et al. Management of oral epithelial dysplasia: a review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103(Suppl S19):e1–12.
55. Ho MW, Risk JM, Woolgar JA, et al. The clinical determinants of malignant transformation in oral epithelial dysplasia. Oral Oncol. 2012;48:969–76.
56. William WN. Oral premalignant lesions. Curr Opin Oncol. 2012;24:205–10.
57. Lodi G, Porter S. Management of potentially malignant disorders: evidence and critique. J Oral Pathol Med. 2008;37:63–9.

Chapter 5
Erythroplakia and Erythroleucoplakia

Lakshminarasimman Parasuraman, Munita Bal, and Prathamesh S. Pai

Introduction

In 1911, the French dermatologist Louis Queyrat described a sharply defined, bright red, glistening velvety precancerous lesion of the glans penis giving rise to the term 'erythroplasia of Queyrat' [1]. In 1978 WHO defined erythroplakia as a "fiery red patch that cannot be characterized clinically or pathologically as any other definable disease" [2]. Bouquot in 1994 updated the definition for erythroplakia as "a chronic red mucosal macule which cannot be given another specific diagnostic name and cannot be attributed to traumatic, vascular, or inflammatory causes" [3]. Erythroplakia and erythroleucoplakia (Speckled leucoplakia) are clinical terms and have no specific histopathologic definition. The correlation between the clinical terminology and pathologic counterparts like hyperplasia, dysplasia and hyperkeratosis are imperfect and that contributes to confusion.

Epidemiology

Relevant studies indicating the incidence and prevalence of oral erythroplakia (OE) are rare but have been seen in <1% of mucosal lesions studied ranging from 0.09% to 0.83% [4, 5]. Most patients fall between 45 and 55 years and have a male preponderance. Buccal mucosa, soft palate, and floor of mouth are the most common sites affected by OE. The floor of mouth in males and combinations of mandibular

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alveolar mucosa and gingival sulcus in females are the most common sites of involvement, while the reason for this predilection is not known [4].

Classification

Clinical variations • Homogenous erythroplakia • Erythroplakia interspersed with patches of leucoplakia • Granular or speckled erythroplakia (speckled leucoplakia)
Microscopic variants 1. Neoplastic • Squamous carcinoma • Carcinoma in situ and less severe forms of epithelial atypia
2. Inflammatory • Candida albicans • Tuberculosis • Histoplasmosis • Others

Aetio-pathogenesis

Tobacco chewing, areca nut and alcohol drinking are strong risk factors for erythroplakia. Human papilloma virus (HPV) especially type-16 has been shown to be associated with oral premalignant disorders [6, 7]. While epidemiological studies suggest evidence for aetiology of OE, its pathogenesis remains a mystery. No conclusive evidence exists either for de novo development of OE or progression from leucoplakia or as a continuum in the multistep carcinogenesis model. However the progression rate of OE to malignancy is higher as compared to leucoplakia [8].

Clinical Features

Usually OE patches are smooth, soft on palpation without induration and have well-defined margin unless transforming into invasive carcinoma. They are rarely multicentric and do not occupy large surface areas unlike leucoplakia. OE gets its reddish appearance due to proximity of the underlying vascular lamina propria brought closer to surface due to the thinning and atrophy of the epithelium. This may not however hold true where the epithelium is fairly thick. Another theory could be that the translucency of a poorly differentiated epithelium is more than normal epithelium leading to the reddish hues [9]. Erythroleucoplakias often get secondarily infected with candida albicans resulting in a red surface either due to inflammation, dysplasia or both [10, 11].

Differential Diagnosis of a Red Oral Mucosa Lesion [12]

OE is a clinical diagnosis but only homogenous erythroplakia has been clearly defined. The terminology used for white, red and mixed lesions is varied and confusing. Relevance of classification in management of these red lesions is a matter of debate. Any suspicious lesion which is erythematous needs histopathological examination to rule out dysplasia/carcinoma in situ changes (Fig. 5.1).

Mucosal diseases Atrophic oral lichen planus Systemic lupus erythematosus Pemphigus Pemphigoid	Oral candidiasis Erythematous candidiasis Generalized candidal erythema Denture-induced stomatitis Histoplasmosis
Bacterial infections Tuberculosis	Others Amelanotic melanoma Hemangioma Telangiectasias, lingual varices Kaposi sarcoma Oral purpura

Pathology

By definition, erythroplakia should not display histopathologic features of any other recognizable condition and is a diagnosis of exclusion [13]. The histologic features of erythroplakia include a wide morphologic spectrum ranging from varying grades of dysplasia and carcinoma in situ (CIS) to invasive squamous cell carcinoma. This

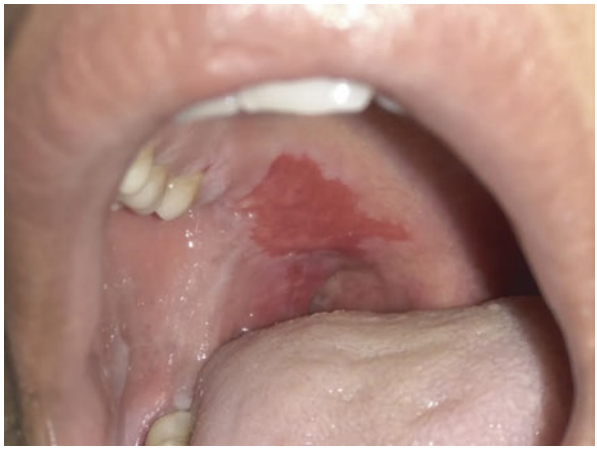

Fig. 5.1 Erythroleucoplakia

implies that erythroplakia harbours epithelial dysplasia as a minimum qualitative alteration.

Histologically, dysplasia entails two types of alterations: architectural and cytologic abnormalities:

- *Architectural alterations* include irregular epithelial stratification, loss of polarity, parabasilar hyperplasia, drop-shaped rete ridges, mitoses in the mid- and upper spinous layer, premature individual cell keratinization (dyskeratosis) and keratin pearls within rete pegs.
- *Cytological alterations* include cellular pleomorphism, nuclear enlargement and pleomorphism, increased nuclear-to-cytoplasmic ratio, prominent nucleoli and nuclear hyperchromasia.

Dysplasia is graded based on quantum of dysplastic change. According to the WHO grading system [13], dysplastic changes confined to the lower third of epithelial thickness are graded as mild, up to two-thirds of the thickness of epithelium as moderate, involving greater than two-thirds but less than the full thickness as severe and occupying the entire thickness of epithelium as CIS, while invasion of the underlying stroma through the basement membrane qualifies as invasive squamous cell carcinoma (Fig. 5.2). Following histologic review, erythroplakia has been found to harbour invasive carcinoma in about 51%, severe dysplasia or CIS in 40% and mild to moderate dysplasia in 9% [12].

One of the challenges in the pathology reporting of dysplasia (especially the lower grades) is the morphological overlap with reactive epithelial atypia imputable to trauma and inflammation. A lack of consensus on the amount and intensity of morphologic alterations engenders subjectivity leading to intra- and interobserver variability [14, 15]. Data from studies based on longitudinally derived specimens are needed to generate objective consensus criteria for grading oral dysplasia.

Role of immunohistochemistry in erythroplakia diagnosis remains limited. Ki-67, a marker of proliferation, is of some utility in cases with ambiguous pathological findings. Increased Ki-67 labelling in the upper two-thirds of the epithelium tends to favour dysplasia over reactive atypia [16, 17]. Some authors have employed other markers such as cytokeratins, CK 8/18, CK19, p53 and p16 [18, 19]. None of the markers is entirely specific or sensitive for dysplasia; however, assist in reinforcing diagnosis in difficult cases.

Molecular Genetics

Genomic instability plays a significant role in the degree of susceptibility of an individual to cancer when exposed to environment-related cancer risk. This genomic instability leads to chromosomal instability either by spontaneous or mutagen induced changes. p53 the “guardian of genome” (tumour suppressor gene) is mutated in 46% of OE specimens often on exons of 6, 5, 8 and 9 of the p53 gene

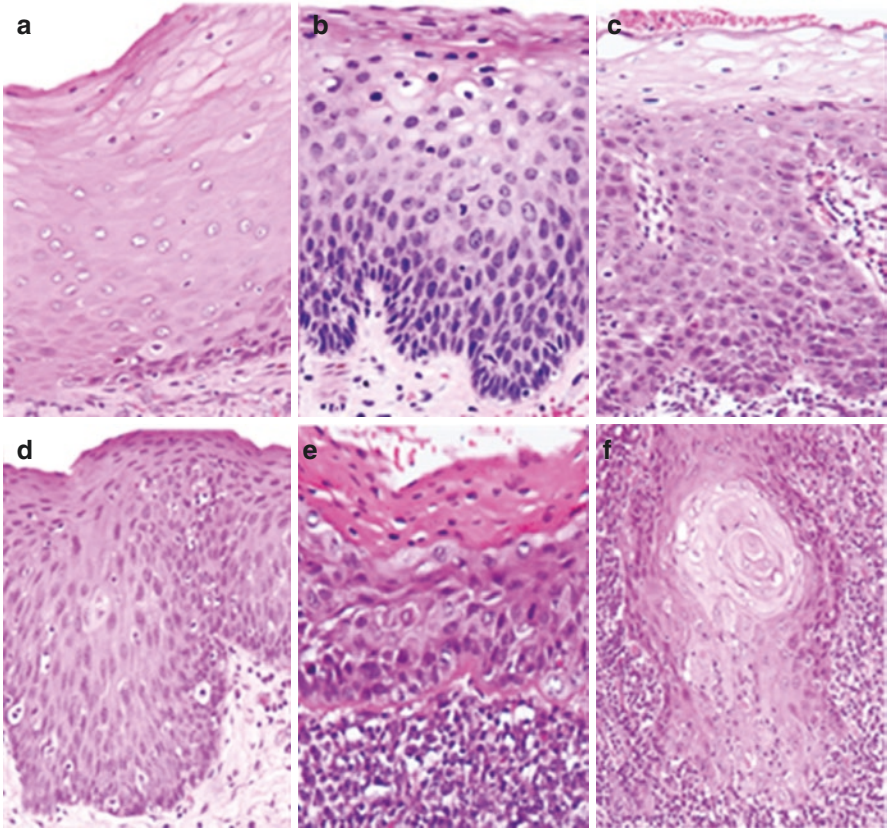

Fig. 5.2 Histopathology spectrum of erythroplakia: (**a**) Normal squamous epithelium; (**b**) mild dysplasia; (**c**) moderate dysplasia; (**d**) severe dysplasia; (**e**) carcinoma in situ; (**f**) invasive squamous cell carcinoma (H&E, 200×)

[20] The damaged DNA moves into mitosis, leading to progressive accumulation of faulty DNA which ultimately results in malignant transformation. Polysomy of chromosomes 7 and 17, LOH, aneuploidy and MSI are also implicated in OE [21]. Various other mutations have been studied in OPMD, but they are not exclusively for erythroplakia.

Management

Early diagnosis of PMD is the key to reduction of mortality and morbidity associated with oral cancer. Cochrane Systematic Reviews have found visual examination by trained health worker to be the best method for early detection of PMD or cancer

in apparently normal individuals when compared to the diagnostic accuracy of various modalities like conventional oral examination (COE), vital rinsing, light-based detection, biomarkers and mouth self-examination (MSE) used alone or in combination [22].

No management guidelines are available but three basic principles should be followed:

- Reducing risk/exposure factors.
- Biopsy/complete removal of the suspicious lesion.
- Follow-up by continuous monitoring may be lifetime.

Erythroplakia may often be not only be an indicator of underlying dysplasia or frank malignancy but also has a higher propensity for malignant transformation in comparison to homogenous leucoplakias. More than a third of the patients have discrepancy between clinical and histological diagnosis, so whenever possible complete removal of the suspicious lesion should be attempted without causing significant morbidity [23]. Large erythroplakic lesions are usually seldom seen and should be subjected to histopathology from a representative area before definitive treatment. Treatment of an oral PMD is usually surgical resection. However whether surgery eliminates the risk of progression to invasive carcinoma or even reduces the incidence is largely unanswered [24].

Adequacy of surgical margins in these lesions is also undefined as also is the issue of recurrence of these lesions. However newer technologies can be used to clear all afflicted tissue. The most promising amongst these is the narrow-band imaging (NBI).

NBI is an advanced optical image system to study the patterns of the submucosal vessels by using the characteristics of light spectrum. It is non-invasive and useful in both diagnosis as well as guiding surgical resection. Twisted elongated and destructive patterns of intra-epithelial microvasculature on NBI images had high correlation with detecting high-grade dysplasia, carcinoma in situ or invasive carcinoma in OE [25].

Erythroplakia may have dysplastic changes well beyond the visible lesion with only subtle or no changes in surface mucosa. This is where NBI comes into play to delineate the abnormal vascular patterns and guide our “true” resection margin [26].

Malignant Transformation Rate

Dysplasia is histopathological evidence of malignant transformation. Shafer and Waldron studied a total of 65 biopsies of OE of homogeneous type and graded the degree of epithelial dysplasia as mild to moderate, severe to carcinoma in situ and carcinoma. Fifty-one percentage were invasive carcinomas, 40% showed carcinoma in situ or severe dysplasia, while the remaining 9% showed mild to moderate dysplasia. This study showed that erythroplakia seems to be at high risk for malignant transformation [4].

Mashberg et al. analysed 500 oral mucosal biopsies of which <2% of asymptomatic white (keratotic) lesions were diagnosed as carcinoma or carcinoma in situ. In contrast asymptomatic erythroplastic components often had at the least in situ changes suggesting that we should consider all erythroplakic lesions as malignant unless proven otherwise [27, 28].

Erythroleucoplakia (speckled leucoplakia) should be considered as a variant of erythroplakia which carries the risk of malignancy between "pure" leukoplakic and "pure" erythroplakic lesions. However there is no documented series reliably stating the annual malignant transformation rate of erythroplakia. Transformation into invasive carcinoma for lesions with severe dysplasia or carcinoma in situ changes ranges from 13% to 50% [29].

Erythroplakias are generally considered to have a higher tendency to undergo malignant transformation than leucoplakias due to its association with varying degrees of dysplasia.

Chemoprevention

OE occurs in far fewer patients in comparison to oral leucoplakia and has never been studied in chemoprevention clinical trials. Retinoids, COX inhibitors, green tea polyphenols, p-53-targeted agents like ONYX-015, thiazolidinediones (PPAR), EFGR inhibitors, blue-green microalgae spirulina, vitamin E, etc. are all studied in OPMD without much success.

Summary

- Erythroplakia constitutes <1% of the oral potential malignant lesions.
- Tobacco chewing and alcohol consumption are etiological factors for erythroplakia.
- Buccal mucosa, soft palate and floor of mouth are the most affected sites in oral cavity often occurring in middle-aged men.
- No defined or proven histopathological features exist for diagnosis of erythroplakia.
- Genomic instability is considered to play a significant role in the degree of susceptibility of an individual to cancer when exposed to environment-related cancer risk.
- Visual examination by trained health worker proved to be the best method for early detection.
- No management guidelines are available.
- Surgery, either by cold knife or by CO_2 laser, is the recommended treatment of choice.
- Follow-up by continuous monitoring may be lifetime.

References

1. Queyrat L. Erythroplasie de gland. Bull Soc Franc Derm Syph. 1911;22:378–82.
2. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36:575–80.
3. Bouquot JE. Oral leukoplakia and erythroplakia: a review and update. Pract Periodontics Aesthet Dent. 1994;6:9–17. quiz 19.
4. Shafer WG, Waldron CA. Erythroplakia of the oral cavity. Cancer. 1975;36:1021–8.
5. Lay KM, Sein K, Myint A, Ko SK, Pindborg JJ. Epidemiologic study of 600 villagers of oral precancerous lesions in Bilugyun: preliminary report. Commun Dent Oral Epidemiol. 1982;10:152–5.
6. Hashibe M, et al. Chewing tobacco, Alcohol, and the risk of erythroplakia mia hashibe cancer epidemiology. Cancer Epidemiol Biomarkers Prev. 2000;9:639–45.
7. Syrjanen S, et al. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis. 2011;17(Suppl 1):58–72. https://doi.org/10.1111/j.1601-0825.2011.01792.
8. Villa A, Villa C, Abati S. Oral cancer and oral erythroplakia: an update and implication for clinicians. Aust Dent J. 2011;56(3):253–6.
9. Shear M. Erythroplakia of the mouth. Int Dent J. 1972;22(4):460–73.
10. Jepsen A, Winther JE. Mycotic infection in oral leukoplakia. Acta Odontol Scand. 1965;23:239–56.
11. Field EA, Field JK, Martin MV. Does candida have a role in oral epithelial neoplasia? J Med Vet Mycol. 1989;27:277–94.
12. Reichart PA, Philipsen HP. Oral erythroplakia: a review. Oral Oncol. 2005;41(6):551–61.
13. WHO Collaborating Centre for Oral Precancerous Lesions. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg. 1978;46:518–39.
14. Pindborg JJ, Reibel J, Holmstrup P. Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. J Oral Pathol. 1985;14:698–708.
15. Abbey LM, Kaugars GE, Gunsolley JC, et al. Intraexaminer and interexaminer reliability in the diagnosis of oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol. 1995;80:188–91.
16. Vered M, Allon I, Dayan D. Maspin, p53, p63, and Ki-67 in epithelial lesions of the tongue: from hyperplasia through dysplasia to carcinoma. J Oral Pathol Med. 2009;38:314–20.
17. Kurokawa H, Matsumoto S, Murata T, Yamashita Y, Tomoyose T, Zhang M, Fukuyama H, Takahashi T. Immunohistochemical study of syndecan-1 down-regulation and the expression of p53 protein or Ki-67 antigen in oral leukoplakia with or without epithelial dysplasia. J Oral Pathol Med. 2003;32:513–21.
18. Kannan S, Balaram P, Chandran GJ, Pillai MR, Mathew B, Nalinakumari KR, Nair MK. Differential expression of cytokeratin proteins during tumour progression in oral mucosa. Epithelial Cell Biol. 1994;3(2):61–9.
19. Wayne S, Robinson RA. Upper aerodigestive tract squamous dysplasia: correlation with p16, p53, pRb, and Ki-67 expression. Arch Pathol Lab Med. 2006;130:1309–14.
20. Qin G-Z, et al. A high prevalence of p53 mutations in pre-malignant oral erythroplakia. Int J Cancer. 1999;80(3):345–8.
21. Hittelman WN, et al. Early genetic changes during upper aerodigestive tract tumorigenesis. J Cell Biochem. 1993;53(S17F):233–6.
22. Walsh T, et al. Clinical assessment to screen for the detection of oral cavity cancer and potentially malignant disorders in apparently healthy adults. Cochrane Database Syst Rev. 2013;(11):CD010173.
23. Onofre MA, et al. Diagnosis of potentially malignant epithelial lesions. Oral Dis. 1997;3(3):148–52.
24. Balasundaram I, et al. Is there any benefit in surgery for potentially malignant disorders of the oral cavity? J Oral Pathol Med. 2014;43(4):239–44.

25. Yang S-W, et al. Clinical characteristics of narrow-band imaging of oral erythroplakia and its correlation with pathology. BMC Cancer. 2015;15(1):1.
26. Tan NCW, et al. Use of narrow band imaging guidance in the management of oral erythroplakia. Br J Oral Maxillofac Surg. 2011;49(6):488–90.
27. Mashberg A, Samit A. Early diagnosis of asymptomatic oraland oropharyngeal squamous cancers. CA Cancer J Clin. 1995;45:328–51.
28. Mashberg A. Erythroplasia: the earliest sign of asymptomatic oral cancer. J Am Dent Assoc. 1978;96:615–20.
29. Bouquot JE, Ephros H. Erythroplakia: the dangerous red mucosa. Pract Periodontics Aesthet Dent. 1995;7:59–67.

Chapter 6
Oral Lichen Planus and the Lichenoid Group of Diseases

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Introduction

Oral cancer is a worldwide health problem with approximately 354.900 new cases estimated for 2018 [1, 2], and oral squamous cell carcinoma (OSCC) is the most common microscopic subtype, accounting for over 90% of all cases [2–4]. Despite some improvements in the understanding of OSCC pathogenesis and in the therapeutic protocols used for treating these patients, the 5-year survival rate of OSCC is still poor, not achieving 50% in most of the surveys in different parts of the globe. Among the factors that may lead to this disappointing number is the advanced stage in which an important percentage of the cases are initially diagnosed [3]. Therefore, not only a better understanding of the molecular mechanism of oral cancer is necessary to improve the prognosis of affected patients but also improvements in the screening strategies for achieving early diagnosis.

Although OSCC may rise de novo from the overlying oral mucosa, it is well known that some cases develop from a preceding oral potentially malignant disorder (OPMD) [5, 6], what may provide clinicians a chance of identifying the first

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stages of the oral carcinogenesis process and, consequently, offer these patients a better surveillance and clinical management [3]. In the oral cavity, there is a range of lesions recognized by carrying a higher potential of malignant transformation, including oral leukoplakia, oral erythroplakia, oral submucous fibrosis, and actinic cheilitis (associated with lip cancer). These disorders are strongly associated with social and cultural habits like tobacco and alcohol use, betel chew, and ultraviolet exposure, respectively [6]. Although the potential of malignant transformation of these disorders is highly variable depending on a great number of factors, especially the grade of epithelial dysplasia present in each case, there seems to be no doubt that they do have a higher risk of transformation [5, 7]. On the other hand, the true potential for malignant transformation of another important group of lesions represented by oral lichen planus (OLP) and oral lichenoid lesions is a matter of controversy for over a century and remains to be fully addressed.

Therefore, in this chapter, we review and discuss the current understanding on the potential for lichen planus and other lichenoid lesions of the oral cavity to undergo malignant transformation. We describe the epidemiological features, possible risk factors, incidence rates, and the main issues that still impair the total acceptance of these lesions as potentially malignant by scientific community.

OLP: From a Chronic Inflammatory Mucocutaneous Disease to a Potentially Malignant Disorder

Overview

Lichen planus was first described in 1869 by Erasmus Wilson and is currently considered a chronic auto-immunologic and inflammatory mucocutaneous disorder that may involve the skin, nails, hair, and mucosas, including the oral cavity, genital, ocular, otic, esophageal, and, less commonly, bladder, nasal, laryngeal, and anal mucosas. It is believed to affect approximately 0.5–2% of the general population. Its exact etiology is still unknown, but possible antigenic challenges in the skin and mucosa in a genetically predisposed patient may represent the initial event to the development of the disease [8–13].

Consistent with its chronic nature, OLP is typically characterized by episodes of emergence and remission, occasionally associated with symptomatic complaints. A number of precipitating factors are hypothesized to play a role in the onset of symptomatic lichen planus, including stressful periods, food ingestion (most frequently citrus and spicy items), systemic illness, poor oral hygiene (dental plaque and calculus are speculated to worsen gingival lichen planus), and others, but their exact mechanisms of action are still unknown [13–16].

The pathogenesis of lichen planus is very complex, and the disease may result from an abnormal CD8+ T-cell-mediated immunoreaction with both antigen-specific and nonspecific mechanisms taking place simultaneously. It is believed that activated T lymphocytes, the main cellular component present in the inflammatory

infiltrate of lichen planus, and the increased production of cytokines lead to an increased intercellular adhesion molecule-1 (ICAM-1) expression by keratinocytes and major histocompatibility complex (MHC) class I antigens, ultimately causing tissue destruction [10, 13, 17, 18]. In addition, a large number of genetic polymorphisms were demonstrated to make individuals more predisposed to develop OLP [13, 18], but no evidence of a familial pattern has been demonstrated.

Clinical Aspects

Clinically, different from cutaneous lichen planus (that usually presents as pruritic and self-limiting polygonal erythematous papules more commonly affecting the ankles, wrists, and the extremities), OLP is considered to evolve in a more chronic manner, only rarely demonstrating spontaneous regression, and also reveals a higher potential to cause local morbidity [16, 19]. Nevertheless, the great majority of OLP is asymptomatic [19], and from 13% to over 20% of the cases are identified by dentists with patients not noticing their mucosal alterations [16, 20]. Consequently, it is reasonable to say that its incidence in the general population is likely to be underestimated.

OLP predominantly affects female patients with a male/female ratios of 1:2 in the great majority of the studies [19, 21, 22], but the reason for this female preponderance remains unclear. The great majority of the affected patients are middle-aged or adult individuals, usually in their fourth to sixth decades of life [19, 23], whereas children and adolescents are only rarely diagnosed with this disease. Interestingly, males are usually younger than females in many studies [20].

Typically, OLP manifests as a multifocal, almost symmetrical, bilateral lesion, and in spite of any mucosal region of the oral cavity that can be affected, the buccal mucosa is by far the most involved site. The tongue, especially the lateral border, but also the dorsum, is the second most involved location in some studies, whereas the gingiva outnumbers the tongue in others. The labial mucosa is also frequently affected, but the floor of the mouth and the palate are less frequently involved [19]. Approximately 10% of the cases manifest isolated in the gingival tissue [13, 18], usually as desquamative gingivitis, and in these cases, diagnosis may be a challenge due to the wide number of differentials and the lack of typical microscopic features when biopsy is done, but at the same time, some authors advocate that these cases represent the most sensible ones to use immunofluorescence as an auxiliary diagnostic tool (Fig. 6.1) [13]. However, it is important to highlight that some cases of desquamative gingivitis may also demonstrate areas containing the more typical whitish striaes, which may facilitate the diagnosis.

OLP may demonstrate a wide range of clinical presentation and is commonly classified into six possible subcategories. The *reticular* pattern is the most common in many studies, presenting as bilateral asymptomatic white lacey lines (Wickham striae), which may, however, be associated with burning sensation when the lateral border of the tongue is affected [20, 23, 24]. The *plaque* type is characterized as hyperkeratotic plaques frequently affecting the dorsum of the tongue. The *atrophic*

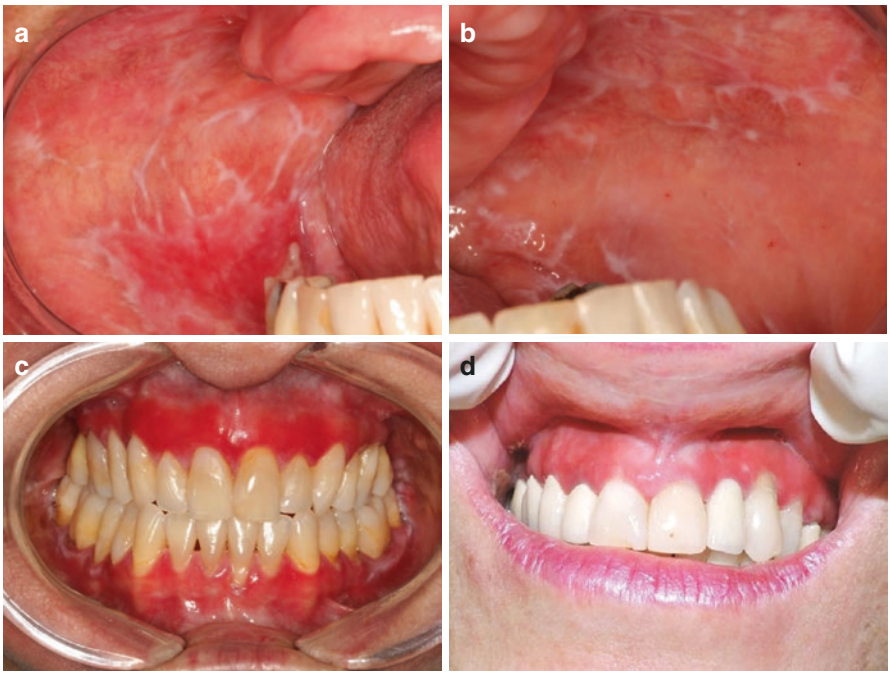

Fig. 6.1 Clinical presentation of oral lichen planus. (**a**, **b**) Bilateral reticular variant of lichen planus also presenting erosive regions in the right side. (**c**, **d**) Gingival lichen planus presenting as desquamative gingivitis

form of the disease demonstrates erythematous areas in the mucosa, and the *erosive* variant is associated with ulcerative lesions of irregular shape and limits. In both subtypes pain is a common finding. The *papular* and the *bullous* variants of OLP are less commonly observed [10]. Because of this variable clinical presentation, misdiagnosis is frequent, and it is also responsible for making the understanding of the malignant potential of OLP more difficult. In an attempt to improve the reproducibility of its clinical diagnosis, some authors classify the lesions in three subtypes: reticular (also including papular and plaque), atrophic, and erosive (also including bullous) [11], whereas some studies classify the lesions in only two groups: white lesions (reticular, popular, and plaque) vs. red lesions (erosive, atrophic, and bullous) [16, 22]. Koebner phenomenon, characterized by clinical manifestations of the disease in areas under traumatic injuries, might be responsible for the clinical presentation of OLP, similar to the one observed in psoriasis. However, this characteristic has not been widely described and is most frequently reported in dermatological literature [13, 25].

Additional to the discomfort of variable intensity, symptomatic patients may also complain of burning sensation, local swelling, dysgeusia, irritation, and bleeding during toothbrushing [21]. Extraoral manifestation in patients affected by OLP, more commonly cutaneous, genital, and nail involvement, is variably observed and was suggested to be present in approximately 6.8–15% of the series [10, 11, 18, 20].

The clinical appearance of OLP is also very important with regard to its malignant transformation, not only by causing problems to achieve the correct diagnosis but also because most of the studies show that malignant degenerations predominantly occur in areas of erosive and atrophic lichen planus [9, 20, 25, 26]. The pathogenic process that makes these variants more susceptible to malignant changes remains unknown, but it has been speculated that the more frequent symptomatic presentation of these subtypes in comparison to reticular lichen planus may facilitate the identification of malignant changes. On the other hand, few reports do not consider the clinical presentation a determinant of transformation, with OSCC arising from both red and white lichen planus [11]. Although pointing toward a more careful follow-up of those patients affected by erosive/atrophic lesions, these controversial results and the lack of better controlled studies regarding the clinical presentation of lichen planus also suggest that all cases need to be closely followed for possible morphologic changes [11].

Hallopeu's manuscript published in 1910 [27] describing a gingival lichen planus transformation into OSCC is considered the first report illustrating the potential for malignant transformation of OLP, which was later classified as a potentially malignant disorder by WHO in 1978 [28, 29]. However, different from what is observed in other potentially malignant disorders, OLP does not seem to be significantly associated with the use of tobacco and alcohol, or with betel chewing [19, 23, 30]. Moreover, in most of the cases where malignant transformation was described, the affected patient was not under the influence of these environmental factors, leading some authors to suggest that carcinomatous changes in OLP could be a natural evolution of the lesion or that such transformation could be associated with other unknown factors [31]. Also in contrast to OSCC arising de novo or from oral leukoplakia where males are more affected, most of the studies observing malignant transformations documented a predominance of females [26, 32, 33]. In addition, although the tongue remains the most affected site by malignant transformations, a high number of lichen planus-derived OSCC affect the buccal mucosa, and some cases originating from the dorsum of the tongue have also been described, a location very rarely affected by OSCC not associated with lichen planus [9, 32–35].

Possible Association with Systemic Diseases and Infections

Lichen planus development has also been associated with a myriad of systemic diseases, including hypertension, diabetes, gastrointestinal peptic disease, hepatic diseases, and others [16, 18, 21]. Although several old studies have documented a possible causative role for diabetes in the onset and malignant transformation of OLP, more recent investigations failed to establish this association, revealing that the frequency of diabetic patients is not significantly different among individuals affected or not by OLP [12, 15, 16].

It is important to remember that patients with different systemic comorbidity are more predisposed to developing lichenoid reactions in the oral cavity as a secondary

undesired side effect of their drugs [11–13]. This clinical scenario is of a high importance because lichenoid lesions that do not fulfill all diagnostic criteria for OLP are frequently included in the samples used to investigate the malignant potential of lichen planus. This group of lesions is also frequently associated with the presence of dental materials, especially amalgam dental restoration, clinically presenting as unilateral and focal lesions ranging from whitish reticular striae to reddish atrophic areas [18]. Microscopically, lichenoid lesions differentiate from OLP due to the presence of other cellular populations like plasma cells, eosinophils, and Langerhans cells in the band-like inflammatory infiltrate, which may also extend deeper in the connective tissue [18, 36].

Although some molecular studies did not find significant differences between OLP and oral lichenoid lesions [37], in a prospective study investigating the malignant potential of 173 patients affected by OLP (62 cases) and oral lichenoid lesions (111 cases), van der Meij et al. [38] observed three malignant changes restricted to the lichenoid group, representing a 219-fold increased risk for these patients. This same group, using a similar sample (192 patients) but with a longer follow-up time, confirmed the initial findings, this time with four patients developing OSCC from a pre-existing oral lichenoid lesion and with a 192-fold increased risk. The authors concluded that patients affected by oral lichenoid lesions, but not by OLP, had an increased potential to undergo malignant transformation, exemplifying the necessity of differentiating both conditions [39].

The importance of liver diseases for OLP pathogenesis has been widely investigated, and controversial results were reported. Some studies reported that the incidence of hepatitis B and C is elevated in patients with OLP [30], whereas other groups claimed that such incidence cannot be considered significantly higher than that observed in the general population. The association between HCV and the potential of OLP to acquire a malignant phenotype has also been speculated, also because active viral particles have been found in epithelial cells of OLP, but this assumption was not fully confirmed and was not observed by others [22]. An important variability in the results regarding the importance of HCV can be observed in literature, and a possible geographical profile may exist, since the most relevant results were obtained in Mediterranean countries and Japan, whereas in America and Britain this association could not be confirmed so far [8, 17, 40]. In addition, HCV infection is commonly associated with cirrhosis, a possible carcinogenic condition, and therefore, the true independent role of HCV in the oral carcinogenesis of lichen planus, if any, cannot be fully addressed.

Another virus whose DNA was reported to be detected in lichen planus is the human papillomavirus (HPV). An evidence linking OLP and HPV infection was provided by Viquier et al. [41] that demonstrated clonal expansion of HPV 16-specific CD8+ T-cell lymphocytes; but previously, Mattila et al. [42] had already identified HPV DNA in 15.9% of their 82 cases investigated, observing the subtypes 6, 11, 16, 31, and 33, and two of the five cases that transformed into OSCC were also positive for HPV. Yildirim et al. [43] also observed 21% of their 65 cases are positive for HPV, whereas Montebugnoli et al. [44] found only four out of 35 cases as positive for HPV (three of them low risk and one high risk) and also demonstrated

that p16 overexpression observed in 26 cases was not associated with the presence of HPV. Recently, in a large systematic review with meta-analysis, Ma et al. [45] reported that OLP patients had a significantly higher prevalence of HPV than normal patients, with an odds ratio ranging from 2.4 to 132.0 depending on the geographic region investigated. The authors, therefore, speculated that HPV may carry an etiological role in the development and possibly in the malignant transformation of OLP. Similar results had also been described previously in the systematic review done by Syrjänen et al. [46] that observed a significant association between HPV and OLP.

Human herpes simplex virus (HHS) and Epstein-Barr virus (EBV) have also been speculated in literature to be associated with OLP [43, 47, 48], but more studies are still necessary to better understand if these viruses would have any etiologic potential or would act as determinants of malignant transformation of OLP. *Candida albicans* infection does not seem to be associated with OLP development; however, it has been implicated in its possible malignant transformation process. It is described that patients with OLP have an increased prevalence of candidal carriage (37% of the cases) possibly as a consequence of the immunomodulatory therapy to which these patients are submitted. The carcinogenic role played by *Candida albicans* is attributed to its capacity of producing the carcinogenic *N*-nitrosobenzylmethylamine, but this theoretic role of the fungus lacks validation [14, 32].

Microscopic Features

The microscopic recognition of OLP may also represent a pitfall in many circumstances. Histologically, the overlying epithelium may demonstrate areas of hyperkeratosis and atrophy, frequently showing elongated epithelial projections (saw-tooth ridges). Also characteristic of lichen planus is the superficial band-like inflammatory infiltrate predominantly composed of T lymphocytes and the liquefactive destruction of the epithelial basal layer, associated with interface mucositis and the presence of degenerated keratinocytes (Civatte bodies). A narrow, eosinophilic, and PAS-positive zone in the basal membrane is frequently described (Fig. 6.2) [38, 49]. Although these features might be considered easily recognized, other lichenoid lesions may also demonstrate similar microscopic findings. In addition, erythematous and erosive lesions are more likely to be diagnosed as nonspecific mucositis, leading pathologists to recommend clinicians to preferentially perform the incisional biopsy in the reticular areas [15, 25]. Immunofluorescence is not very specific, but fibrin and shaggy fibrinogen linear deposit at the basement membrane zone may be useful in some cases [13, 50].

Differentiating OLP from oral leukoplakia may be difficult in some cases, and this diagnostic overlap has long been considered the main bias present in studies attempting to determine the malignant potential of the lesion. As an example of this diagnostic concern, Krutchkoff and Eisenberg in 1985 proposed the term lichenoid

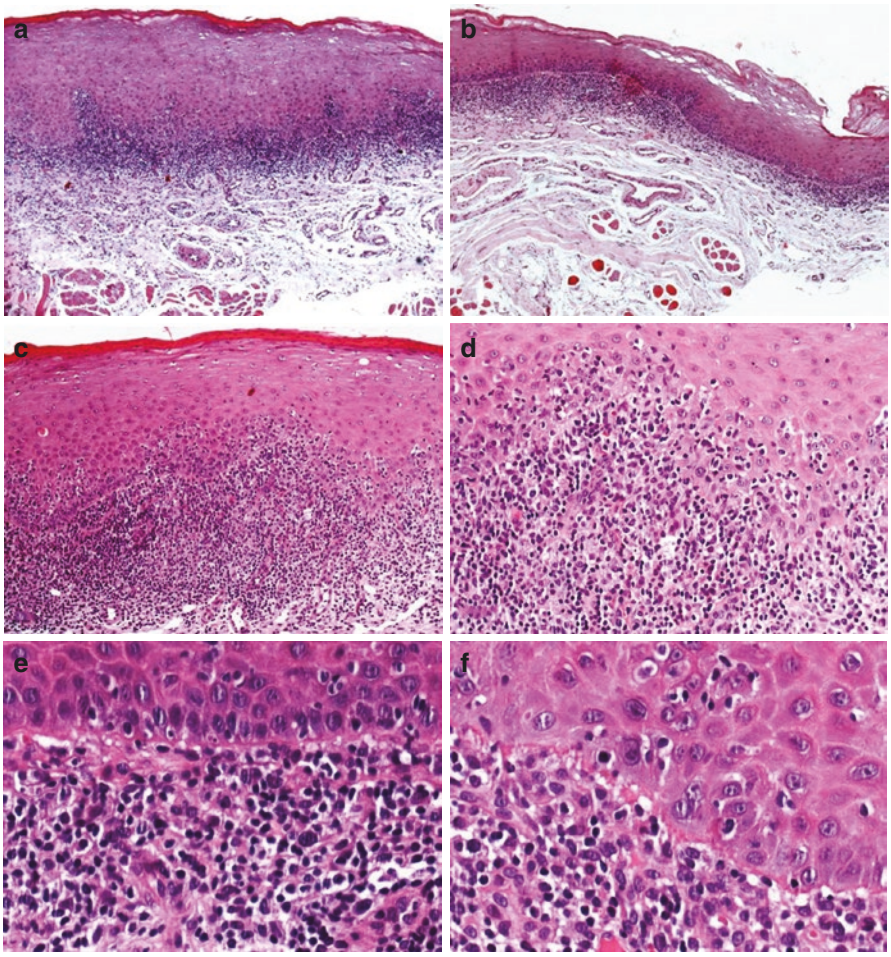

Fig. 6.2 Microscopic findings of oral lichen planus. (**a**) Acanthotic epithelium with a band-like inflammatory infiltrate (H&E; 50×). (**b**) The epithelium demonstrates areas of atrophy (H&E; 50×). (**c**, **d**) Liquefactive destruction of the epithelial basal layer (H&E; 100× and 200×, respectively). (**e**) Chronic inflammatory infiltrate predominantly composed of lymphocytes (H&E; 400×). (**f**) A narrow, eosinophilic zone in the basal membrane is seen (H&E; 400×)

dysplasia that defined an oral leukoplakia containing different grades of epithelial dysplasia in its microscopic features, but also associated with a lichenoid infiltrate [51]. Because epithelial dysplasia is considered the most important parameter to determine the malignant potential of a given disorder, it was established that when evaluating OLP for such potential, cases consistent with lichenoid dysplasia must be excluded; therefore, OLP with any degree of epithelial dysplasia cannot be included in these samples [52]. However, several studies still include lichenoid dysplasia in their studies. Bornstein et al. [53] observed that of their four cases with malignant transformation, three had some epithelial dysplasia, while all three cases

with transformation reported by Pakfetrat et al. [24] also demonstrated mild dysplasia. The diagnostic overlap between OLP and lichenoid dysplasia is even more commonly observed in those studies investigating all potentially malignant disorders of the oral cavity, where authors usually do not review the microscopic aspects of their samples and do not exclude OLP with dysplasia, an issue that has also been observed in many studies investigating the molecular features of OLP [54, 55].

Another microscopic issue that may significantly impact the interpretation of an important number of currently available studies on the malignant potential of OLP is the definition of proliferative verrucous leukoplakia (PVL). This entity is known to carry a high potential to undergo malignant change, and original reports dating back to the 1990s supported that 100% of cases would give rise to OSCC in a specific point of time. In addition to the similar clinical presentation between early stages of PVL (characterized by multiple hyperkeratotic plaques with or without a verrucous surface) and the plaque variant of OLP, Lopes et al. [56] have recently demonstrated that several cases that were later consistent with the diagnosis of PVL initially presented clinical features resembling reticular oral lichenoid reactions. Therefore, when dealing with malignant transformation of lichen planus, especially those with multifocal transformations [8, 57], diagnosticians must consider the possibility of PVL and a careful review of microscopic aspects should be done.

In the context of transplanted patients, diagnosticians must rule out the possibility of graft-versus-host disease (GVHD), which may demonstrate similar clinical and microscopic features with lichen planus and has been associated with the development of OSCC [10, 58]. Moreover, oral manifestation of discoid lupus erythematosus must also be differentiated from OLP because both entities may reveal erythematous lesions with coexistence of reticular striae and a similar microscopic appearance [25]. Like in GVHD, discoid lupus erythematosus has also been associated with a possible malignant potential [59]; nevertheless, the prediction role of both diseases for oral cancer development remains to be fully established.

Diagnostic Criteria and Pitfalls for Malignant Transformation Analysis

According to the WHO, the appropriate diagnosis of OLP demands a simultaneous identification of the abovementioned clinical and microscopic parameters. However, van der Meij et al. [50] demonstrated a very high inter- and intra-observer variability in the clinical and histological assessment of OLP based on the WHO definitions, and also because of the diagnostic overlaps with lichenoid dysplasias cited before, the authors recommended a modification in the diagnostic criteria for OLP. From the clinical point of view, the authors suggested that the disease should be characterized by a bilateral, more or less symmetrical lesions, with a whitish to grayish lacey-like network consistent with the reticular pattern of lichen planus, and when atrophic, erosive, bullous, or plaque variants were considered, at least a focus

of the reticular pattern has to be present elsewhere in the oral mucosa. In the absence of one of these criteria, these lesions must be considered "clinically compatible with OLP." Simultaneously, the microscopic findings of OLP have to reveal the presence of a well-defined band-like inflammatory infiltrated predominantly lymphocytic, restricted to the superficial area of the connective tissue, signs of basal cell degeneration, and absence of epithelial dysplasia. If the microscopic features of a given case do not clearly present these features, the report must be "histopathologically compatible with OLP." According to the authors, only when all these clinical and microscopic features were present, a final diagnosis of OLP can be reported, whereas the term oral lichenoid lesion should be preferred in the absence of any parameter.

By adopting this modified criterion, a more rigorous methodology for investigating malignant transformation in OLP is applied and more reliable results are obtained. In addition to these diagnostic recommendations, it has also been advised that a minimum follow-up time between initial diagnosis of OLP and the first evidence of malignant change must be of 6 months in order to exclude those cases with possible concomitant presentations, although some authors have advocated the use of 24 months as latency period [30]. Furthermore, to accept a progression to oral cancer, such change must occur in the same site as the initial lesion, not necessarily in the same location from where the biopsy was taken. Different authors have also recommended that patients evaluated for malignant transformation were not exposed to known carcinogenic factors, like tobacco and alcohol [53].

After an extensive literature review on malignant change of OLP, with data being summarized in Table 6.1, we observed that the percentage of transformation ranges from 0.0% to over 5.56%, exemplifying that such event is very rare. However, the overall follow-up time has also been a limiting factor for most of the available studies, since different periods were reported and some of them with a short follow-up time. Consequently, the transformation rates obtained by dividing the number of malignant changes by the total number of patients in the sample may be significantly influenced [60]. To minimize this limitation, it is recommended that the incidence per time is provided; however, this data is frequently absent in the studies, and the appropriate comparison between different analyses becomes very unlikely to be done [52]. When the frequency of transformations per year is shown, an annual transformation rate ranging from 0.00% to 0.69% is observed, confirming that any possible malignant change in OLP is very unusual.

A number of recommendations have been provided in the last 30 years to minimize or exclude confusing factors that for a long time impaired authors to obtain a more reliable data on the malignant potential of OLP, most of them related to diagnostic criteria. Consequently, some recent papers have provided important findings on this topic. However, most of our knowledge remains based on retrospective and occasional simple observational prospective studies, while well-controlled, blinded, clinical trials are still absent in literature [60], possibly because of the difficulties to organize a large sample of an uncommon lesion during a long period of time, what would demand an expensive financial and technical investment.

Table 6.1 Summary of the number and percentage observed in literature of oral lichen planus demonstrating malignant transformation

Authors	Country	No. cases	No. and % of transformations	Follow-up (months)	Time to transformation (months)	Methodology
Wang et al. [3]	Taiwan	381	2 (0.52%)	NA	8.07	Retrospective
Budimir et al. [19]	Croatia	563	4 (0.70%)	NA	91.2	Retrospective
Bardellini et al. [12]	Italy	204	2 (0.98%)	NA	50	Retrospective
Kaplan et al. [11]	Israel	171	6 (3.50%)	Mean of 51.6	NA	Retrospective
Shen et al. [31]	China	518	5 (0.96%)	From 6 to 258	70.4	Retrospective
Brzak et al. [23]	Croatia	537	2 (0.37%)	NA	NA	Retrospective
Bombeccari et al. [57]	Italy	327	8 (2.40%)	Mean of 82	39.3	Prospective
Warnakulasuriya et al. [6]	UK	607	6 (1.00%)	NA	NA	Retrospective
Torrente-Castells et al. [88]	Spain	65	2 (3.07%)	Mean of 18.2	12[a]	Retrospective
Fang et al. [33]	China	2119	23 (1.08%)	NA	16	Retrospective
Bermejo-Fenoll et al. [22]	Spain	550	8 (0.90%)	NA	NA	Retrospective
Pakfetrat et al. [24]	Iran	420	3 (0.07%)	NA	26.0	Retrospective
Carbone et al. [16]	Italy	808	15 (1.85%)	Mean of 47.4 and 44.8[b]	52.3	Retrospective
Hsue et al. [5]	Taiwan	143	3 (2.10%)	NA	14.7	Retrospective
Van der Meij et al. [39]	The Netherlands	67	0 (0.00%)	Mean of 55.9	Not applied	Prospective
Ingafou et al. [20]	UK	690	13 (1.90%)	NA	84	Retrospective
Bornstein et al. [53]	Switzerland	138	1 (0.71%)	Mean of 44	NA	Retrospective
Xue et al. [15]	China	674	4 (0.59%)	NA	108	Retrospective
Gandolfo et al. [30]	Italy	402	9 (2.23%)	Mean of 58.8	44.5	Prospective
Lanfranchi et a. [83]	Argentina	719	32 (4.45%)	NA	20.8	Retrospective

(continued)

Table 6.1 (continued)

Authors	Country	No. cases	No. and % of transformations	Follow-up (months)	Time to transformation (months)	Methodology
van der Meij et al.[c] [38, 50]	The Netherlands	62	0 (0.00%)	Mean of 31.9	Not applied	Prospective
Eisen et al. [25]	USA	723	6 (0.80%)	Mean of 54	41	Retrospective
Mignogna et al. [35]	Italy	578	24 (4.15%)	NA	31.2	Retrospective
Mignogna et al. [9]	Italy	502	18 (3.70%)	From 4 to 12	NA	Retrospective
Rajentheran et al. [34]	UK	832	7 (0.80%)	Mean of 132	72.0	Retrospective
Lo Muzio et al. [8]	Italy	263	14 (5.32%)	NA	80.5	Retrospective
Markopoulos et al. [84]	Greece	326	4 (1.30%)	Mean of 57.6	78	Prospective
Brown et al. [21]	USA	193	0 (0.00%)	NA	Not applied	Retrospective
Silverman et al. [86]	USA	214	5 (2.30%)	Mean of 90	108	Prospective
Sigurgeirsson et al. [89]	Sweden	2071	8 (0.40%)	Mean of 119	NA	Retrospective
Salem et al. [85]	Saudi Arabia	72	4 (5.56%)	Mean of 38	NA	Retrospective
Holmstrup et al. [32]	Denmark	611	9 (1.50%)	Mean of 90	121.2	Prospective
Silverman et al. [87]	USA	570	7 (1.20%)	Mean of 66	40.8	Prospective

[a]One patient developed OSCC after 24 months of follow-up, and the other patient had an OSCC simultaneous to oral lichen planus. NA: Not available
[b]Median follow-up time for males and females, respectively
[c]van der Meij et al. [50] investigated 173 patients in total, but only 62 were classified as OLP, whereas 111 were diagnosed as oral lichenoid lesion, from which three developed an OSCC. NA: Not available

As a consequence of the limitations frequently observed in numerous surveys, after a literature review in the period 1950–1976, Krutchkoff et al. [61] accepted only 15 out of 223 (7%) cases as demonstrating a true malignant transformation of an OLP, explaining that main factors leading to unacceptable cases were associated with non-convincing initial diagnosis of lichen planus, lack of data on risk factors like the use of tobacco and alcohol, and OSCC arising from sites other than those where lichen planus was initially diagnosed. Similarly, van der Meij et al. [62] accepted only 33 of 98 (34%) reported cases from 1977 to 1999, also supporting the low power of the results available until that time. However, most of the authors' complaints are still found in recent studies, even after the strict recommendations described above.

Biological Behavior of OSCC Developing from Pre-existing OLP

Another topic that remains to be fully investigated is the clinical behavior of OSCC arising from pre-existing OLP. Although some authors affirm that these epithelial malignancies would behave more aggressively, with higher rates of lymph node metastases than oral cancer developing de novo or from oral leukoplakias, even with lower histological grades, most of the studies report the opposite, with less aggressive tumors and high survival rates for affected patients [57, 63]. In addition, an important amount of the cases originating from lichen planus were diagnosed as in situ carcinoma or grade I carcinomas, what may be a consequence of a more strict follow-up schemes to which OLP patients are submitted, with more frequent professional visits during the year [35, 63]. Some series have also documented the development of verrucous carcinomas that biologically carries a less aggressive and less infiltrative potential, what could indicate that malignancies originating from OLP may have inherited a lower aggressiveness. However, because of the rarity of malignant changes in these chronic lesions, follow-up studies of malignancies developing from OLP are very rare [63].

Treatment of OLP and Its Possible Association with Malignant Change

The main objective in OLP treatment is to ensure that the symptoms of the disease are improved [64]. Therapeutic management of this disease is based on the use of immunomodulatory drugs, but the clinical presentation of the disease and the intensity of symptoms will determine what drugs, concentrations, and doses will be used in each case. Because most of the lesions are confined to the oral cavity, topical corticosteroids are the gold standard protocol for these patients especially the use of

clobetasol propionate ointment 0.05% applied over the affected areas of the oral mucosa. Cyclosporine 3%, fluocinonide ointment 0.05%, retinoic acid gel 0.025%, and betamethasone rinses are also variably reported in literature. However, recalcitrant and systemically widespread lesions usually require systemic therapy, which includes tacrolimus, triamcinolone, and prednisone use in variable protocols [11, 16, 30].

Patients affected by OLP are also predisposed to fungal infection due to the chronic use of corticosteroids; therefore, many authors advocate the concomitant antimycotic prophylactic schemes that include the use of miconazole gel, fluconazole rinses, nystatin, and 0.12% chlorhexidine [12, 14, 16].

The great majority of the cases is appropriately managed with these therapeutic schemes, and symptomatic complaints are frequently relieved in a short period of time. However, some authors support the notion that the immunomodulatory therapy itself would be responsible for increasing the risk for malignant transformation of OLP. According to some authors, this therapy could depress local cell-mediated immunity and promote the progression of malignant development [57, 64]. Moreover, it has also been stated that corticosteroid therapy could not only hasten this process, but it would also do so with reduced symptoms. Although theoretically supported by some, this possibility has not found scientific evidences that could support the abandon of its use, and other authors failed to observe any significant association between corticosteroids use and a higher frequency of malignant changes [12, 16].

Molecular Events That May Support the Potentially Malignant Definition of OLP

Following the clinical and microscopic evidences that supported OLP as a potential malignant disorder, an uncountable number of studies have been published recently aiming to determine the molecular basis that would make this lesion more susceptible to undergo a carcinomatous degeneration. Hence, the expression of many genes and proteins previously demonstrated to be important for OSCC pathogenesis was investigated in the context of lichen planus. Alves et al. [65] demonstrated that the cell cycle regulators p53 and MDM2 were overexpressed in OLP, with a similar pattern to the observed in oral leukoplakias with epithelial dysplasia and in OSCC, suggesting the existence of a favorable environment for malignant transformation in lichen planus. In this line, Gonzáles-Moles et al. [58] using a panel of markers that included p53, p21, caspase-3, bcl-2, and Ki67 demonstrated that cell apoptosis would be infrequent or absent in OLP, therefore creating a suitable substrate for malignant degeneration. Poomsawat et al. [66] demonstrated that cdk4 and p16, also important molecules in the cell cycle control, were overexpressed in OLP than in normal mucosa, leading the authors to hypothesize that the lesional epithelial cells would be in a hyperproliferative state.

The nuclear factor-κB (NF-κB) protein, an important molecular in the regulation of normal inflammatory and immunologic processes and also known to be involved in the pathogenesis of a large number of human neoplasms, including OSCC, has also been extensively investigated in the OLP context. Zhou et al. [67] demonstrated that this protein and TNF-α were both upregulated in OLP than in normal tissue, also correlating with the clinical appearance of the lesion (higher expression in atrophic/erosive forms than in reticular forms). Santoro et al. [68] also investigated NF-κB in the lichen planus context and demonstrated that the protein is overexpressed in oral cavity lesions than in cutaneous disease and was associated with the amount of cytotoxic cells in the inflammatory infiltrate. These and other studies show the importance of NF-κB for lichen planus pathogenesis and malignant potential, suggesting that NF-κB inhibitors would be potential drugs in their management.

Some molecular markers have also been investigated regarding their predictive potential for malignant transformation. Segura et al. [69] investigated the importance of the proto-oncogene MYC for OLP transformation using in situ hybridization and immunohistochemistry in 17 OLP that undergone malignant transformation, in 11 OSCC developed from the previous group, and in 13 OLP that did not develop oral cancer. The authors observed that lesions with progression to oral cancer have MYC gains and c-Myc overexpression, suggesting that MYC status would be a useful parameter to determine which patient is at risk for transformation. Using a similar approach, Xu et al. [70] also demonstrated that aldehyde dehydrogenase 1 (ALDH1), a stem cell marker, would be a significant predictor of malignant transformation in OLP by demonstrating a higher expression of this marker in cases with malignant change when compared to those that did not acquire any malignant phenotype.

Considering the relevance of DNA ploidy status as a potential biomarker for oral carcinogenesis described in different oral malignant and potentially malignant lesions [71, 72], its importance in the OLP context has also been addressed. Pentenero et al. [73] investigated the DNA status of 77 patients affected by OLP by flow cytometry and found only two cases to be aneuploidy, concluding that DNA aneuploidy in OLP is less frequent than in other oral potentially malignant disorders. Similar results were obtained by Acha-Sagredo et al. [74] that observed no aneuploidy in 40 patients affected by OLP investigated by cytology and image cytometry. On the other hand, Maraki et al. [75] supported the diagnostic sensitivity of cytology associated with DNA ploidy analysis in the identification of malignant changes of OLP, reporting that by demonstrating aneuploidy in two cases, the technique was able to recognize the only case associated with malignant changes in their sample. Sperandio et al. [76] who investigated OLP with and without carcinomatous transformation demonstrated that DNA status analysis predicted transformation in 36.4% of their sample, which, according to the authors, could not be achieved using conventional histology, supporting the use of DNA ploidy analysis for lichen planus malignant potential. Moreover, Hosni et al. [77] observed that atrophic-erosive variants of OLP would carry more aneuploidy cases than reticular variant.

Other genetic investigations have also been conducted in OLP. Accurso et al. [78] investigated the loss of heterozygosity (LOH) and the microsatellite instability at three tumor suppressor loci of patients affected by OLP, other potentially malignant lesion and OSCC. The authors demonstrated that the profile found in lichen planus cases was more similar to a normal mucosa than with dysplastic and neoplastic epithelium, arguing that if strict criteria are used for the diagnosis of lichen planus, it more likely does not demonstrate a potentially malignant phenotype. Similar results have been previously described by Zhang et al. [79]. Investigating the allelic loss at 9 loci in chromosomes 3p, 9p, and 17p, the authors observed LOH in 6% of OLP, 14% of reactive lesions, 40% of mild dysplasia, 46% of moderate dysplasia, 81% of severe dysplasia/carcinoma in situ, and 91% of OSCC, also demonstrating that OLP had a profile more similar to reactive tissues than with dysplastic or neoplastic epithelia. However, more recently, Rodrigues et al. [37] who investigated chromosomes 9p, 11q, and 17p observed that LOH occurred similarly in OLP and lichenoid lesions, but higher than in normal oral mucosa, which demonstrated no LOH.

The expression pattern of microRNA (miRNA) has also been investigated in OLP. Using 30 cases of OLP, Nylander et al. [80] observed that 15 miRNAs demonstrated a different expression than control group, and some of them were also known to be associated with OSCC pathogenesis. Danielsson et al. [81] also revealed altered expression of miRNAs (miR-21, miR-125b, and miR-203) in OLP than in normal mucosa. Many other authors have already attempted to identify alterations in the expression level of miRNAs in OLP, what may be an important factor in the development of the lesion. Moreover, Dang et al. [82] investigated the methylation status of miRNA-137 promoter as a determinant of malignant transformation of OLP. The authors observed that 0% of normal tissue, 35% of OLP, and 58.3% of OSCC demonstrated methylation levels, leading the authors to speculate that miRNA-137 methylation would carry a predictive potential for malignant transformation of OLP.

Conclusion

According to available articles that investigated patients affected by OLP, it seems that only a very small rate of malignant transformation may occur in these chronic autoimmune disorders. However, the difficult reproducibility of lichen planus diagnosis, the short and irregular follow-up periods, the simultaneous use of risk factors for oral cancer, and the lack of a well-controlled prospective clinical study are some limitations that impair that any strong conclusion on the real potential for carcinomatous change of OLP is obtained. Moreover, these methodological limitations also avoid that possible predictive factors for OSCC development are found. Therefore, the main objective of future studies must be to obtain large samples of cases strictly selected based on current clinical and morphological criteria for diagnosing OLP, so that more reliable data can be provided and the controversial discussion on

malignant transformation of OLP that already takes over a century can be better clarified. New biomarkers may also improve the diagnosis of OLP and better differentiate this lesion from its main differential diagnoses.

References

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2018. in press; https://doi.org/10.1002/ijc.31937.
2. Curado MP, Johnson NW, Kerr AR, Silva DRM, Lanfranchi H, Pereira DL, et al. Oral and oropharynx cancer in South America: incidence, mortality trends and gaps in public databases as presented to the Global Oral Cancer Forum. Transl Res Oral Oncol. 2016;1:1–7.
3. Wang YY, Tail YH, Wang WC, Chen CY, Kao YH, Chen YK, et al. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health. 2014;14:99.
4. Pontes FS, Carneiro JT Jr, Fonseca FP, da Silva TS, Pontes HA, Pinto Ddos S Jr. Squamous cell carcinoma of the tongue and floor of the mouth: analysis of survival rate and independent prognostic factors in the Amazon region. J Craniofac Surg. 2011;22:925–30.
5. Hsue SS, Wang WC, Chen CH, Lin CC, Chen YK, Lin LM. Malignant transformation in 1458 patients with potentially malignant oral mucosal disorders: a follow-up study based in a Taiwanese hospital. J Oral Pathol Med. 2007;36:25–9.
6. Warnakulasuriya S, Kovacevic T, Madden P, Coupland VH, Sperandio M, Odell E, et al. Factors predicting malignant transformation in oral potentially malignant disorders among patients accrued over a 10-year period in South East England. J Oral Pathol Med. 2011;40:677–83.
7. van der Waal I. Oral potentially malignant disorders: is malignant transformation predictable and preventable? Med Oral Patol Oral Cir Bucal. 2014;19:e386–90.
8. Lo Muzio L, Mignogna MD, Favia G, Procaccini M, Testa NF, Bucci E. The possible association between oral lichen planus and oral squamous cell carcinoma: a clinical evaluation on 14 cases and a review of the literature. Oral Oncol. 1998;34:239–46.
9. Mignogna MD, Lo Muzio L, Lo Russo L, Fedele S, Ruoppo E, Bucci E. Clinical guidelines in early detection of oral squamous cell carcinoma arising in oral lichen planus: a 5-year experience. Oral Oncol. 2001;37:262–7.
10. Farhi D, Dupin N. Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: facts and controversies. Clin Dermatol. 2010;28:100–8.
11. Kaplan I, Ventura-Sharabi Y, Gal G, Calderon S, Anavi Y. The dynamics of oral lichen planus: a retrospective clinicopathological study. Head Neck Pathol. 2012;6:178–83.
12. Bardellini E, Amadori F, Flocchini P, Bonadeo S, Majorana A. Clinicopathological features and malignant transformation of oral lichen planus: a 12-years retrospective study. Acta Odontol Scand. 2013;71:834–40.
13. Olson MA, Rogers RS III, Bruce AJ. Oral lichen planus. Clin Dermatol. 2016;34:495–504.
14. Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K. Current controversies in oral lichen planus: report of an international consensus meeting. Part 2. Clinical management and malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100:164–78.
15. Xue JL, Fan MW, Wang SZ, Chen XM, Li Y, Wang L. A clinical study of 674 patients with oral lichen planus in China. J Oral Pathol Med. 2005;34:467–72.
16. Carbone M, Arduino PG, Carrozzo M, Gandolfo S, Argiolas MR, Bertolusso G, et al. Course of oral lichen planus: a retrospective study of 808 northern Italian patients. Oral Dis. 2009;15:235–43.

17. Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K. Current controversies in oral lichen planus: report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100:40–51.
18. Scully C, Carrozzo M. Oral mucosal disease: lichen planus. Br J Oral Maxillofac Surg. 2008;46:15–21.
19. Budimir V, Richter I, Andabak-Rogulj A, Vučićević-Boras V, Budimir J, Brailo V. Oral lichen planus - retrospective study of 563 Croatian patients. Med Oral Patol Oral Cir Bucal. 2014;19:e255–60.
20. Ingafou M, Leao JC, Porter SR, Scully C. Oral lichen planus: a retrospective study of 690 British patients. Oral Dis. 2006;12:463–8.
21. Brown RS, Bottomley WK, Puente E, Lavigne GJ. A retrospective evaluation of 193 patients with oral lichen planus. J Oral Pathol Med. 1993;22:69–72.
22. Bermejo-Fenoll A, Sanchez-Siles M, López-Jornet P, Camacho-Alonso F, Salazar-Sanchez N. Premalignant nature of oral lichen planus. A retrospective study of 550 oral lichen planus patients from south-eastern Spain. Oral Oncol. 2009;45:e54–6.
23. Brzak BL, Mravak-Stipeti M, Canjuga I, Baricevic M, Balicevic D, Sikora M, et al. The frequency and malignant transformation rate of oral lichen planus and leukoplakia – a retrospective study. Coll Antropol. 2012;36:773–7.
24. Pakfetrat A, Javadzadeh-Bolouri A, Basir-Shabestari S, Falaki F. Oral lichen planus: a retrospective study of 420 Iranian patients. Med Oral Patol Oral Cir Bucal. 2009;14:E315–8.
25. Eisen D. The clinical features, malignant potential, and systemic associations of oral lichen planus: a study of 723 patients. J Am Acad Dermatol. 2002;46:207–14.
26. Hietanen J, Paasonen MR, Kuhlefelt M, Malmström M. A retrospective study of oral lichen planus patients with concurrent or subsequent development of malignancy. Oral Oncol. 1999;35:278–82.
27. Hallopeau H. Sur un cas de lichen de Wilson gingival avec neoplasia voisine dans la région maxillaire. Bull Soc Fr Dermatol Syphiligr. 1910;17:32.
28. WHO Collaborating Centre for Oral Precancerous Lesions. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg. 1978;46:518–39.
29. Gale N, Pilch BZ, Sidransky D. Epithelial precursor lesions. In: Barnes L, Evenson JW, Reichart P, Sidransky D, editors. Pathology & genetics. Head and neck tumours. Lyon: IARC; 2005. p. 177–81.
30. Gandolfo S, Richiardi L, Carrozzo M, Broccoletti R, Carbone M, Pagano M, et al. Risk of oral squamous cell carcinoma in 402 patients with oral lichen planus: a follow-up study in an Italian population. Oral Oncol. 2004;40:77–83.
31. Shen ZY, Liu W, Zhu LK, Feng JQ, Tang GY, Zhou ZT. A retrospective clinicopathological study on oral lichen planus and malignant transformation: analysis of 518 cases. Med Oral Patol Oral Cir Bucal. 2012;17:e943–7.
32. Holmstrup P, Thorn JJ, Rindum J, Pindborg JJ. Malignant development of lichen planus-affected oral mucosa. J Oral Pathol. 1988;17:219–25.
33. Fang M, Zhang W, Chen Y, He Z. Malignant transformation of oral lichen planus: a retrospective study of 23 cases. Quintessence Int. 2009;40:235–42.
34. Rajentheran R, McLean NR, Kelly CG, Reed MF, Nolan A. Malignant transformation of oral lichen planus. Eur J Surg Oncol. 1999;25:520–3.
35. Mignogna MD, Lo Russo L, Fedele S, Ruoppo E, Califano L, Lo Muzio L. Clinical behaviour of malignant transforming oral lichen planus. Eur J Surg Oncol. 2002;28:838–43.
36. Gueiros LA, Gondak R, Jorge-Júnior J, Coletta RD, Carvalho AA, Leão JC, et al. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;113:661–6.
37. Rodrigues LN, Sousa SF, Silva RC, Abreu MH, Pires FR, Mesquita RA, et al. Allelic loss in amalgam-associated oral lichenoid lesions compared to oral lichen planus and mucosa. Oral Dis. 2017;23:471.

38. van der Meij EH, Schepman KP, van der Waal I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96:164–71.
39. van der Meij EH, Mast H, van der Waal I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective five-year follow-up study of 192 patients. Oral Oncol. 2007;43:742–8.
40. Ingafou M, Porter SR, Scully C, Teo CG. No evidence of HCV infection or liver disease in British patients with oral lichen planus. Int J Oral Maxillofac Surg. 1998;27:65–6.
41. Viguier M, Bachelez H, Poirier B, Aubin F, Touzé A, Carmagnat M, Francès C, Gougeon ML, Fazilleau N. Peripheral and local human papillomavirus 16-specific CD8+ T-cell expansions characterize erosive oral lichen planus. J Invest Dermatol. 2015;135:418–24.
42. Mattila R, Rautava J, Syrjänen S. Human papillomavirus in oral atrophic lichen planus lesions. Oral Oncol. 2012;48:980–4.
43. Yildirim B, Sengüven B, Demir C. Prevalence of herpes simplex, Epstein Barr and human papilloma viruses in oral lichen planus. Med Oral Patol Oral Cir Bucal. 2011;16:e170–4.
44. Montebugnoli L, Gissi DB, Scapoli L, Palmieri A, Morandi L, Manelli I, et al. p16(INK4) expression is not associated with human papillomavirus in oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;118:694–702.
45. Ma J, Zhang J, Zhang Y, Lv T, Liu J. The Magnitude of the association between human papillomavirus and oral lichen planus: a meta-analysis. PLoS One. 2016;11:e0161339.
46. Syrjänen S, Lodi G, von Bültzingslöwen I, Aliko A, Arduino P, Campisi G, et al. Human papillomaviruses in oral carcinoma and oral potentially malignant disorders: a systematic review. Oral Dis. 2011;17(Suppl 1):58–72.
47. Sand LP, Jalouli J, Larsson PA, Hirsch JM. Prevalence of Epstein-Barr virus in oral squamous cell carcinoma, oral lichen planus, and normal oral mucosa. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;93:586–92.
48. Kis A, Fehér E, Gáll T, Tar I, Boda R, Tóth ED, et al. Epstein-Barr virus prevalence in oral squamous cell cancer and in potentially malignant oral disorders in an eastern Hungarian population. Eur J Oral Sci. 2009;117:536–40.
49. Agha-Hosseini F, Sheykhbahaei N, SadrZadeh-Afshar MS. Evaluation of potential risk factors that contribute to malignant transformation of oral lichen planus: a literature review. J Contemp Dent Pract. 2016;17:692–701.
50. van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med. 2003;32:507–12.
51. Fitzpatrick SG, Honda KS, Sattar A, Hirsch SA. Histologic lichenoid features in oral dysplasia and squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117:511–20.
52. Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: a systematic review. J Am Dent Assoc. 2014;145(1):45–56.
53. Bornstein MM, Kalas L, Lemp S, Altermatt HJ, Rees TD, Buser D. Oral lichen planus and malignant transformation: a retrospective follow-up study of clinical and histopathologic data. Quintessence Int. 2006;37:261–71.
54. Rhodus NL, Cheng B, Myers S, Miller L, Ho V, Ondrey F. The feasibility of monitoring NF-kB associated cytokines: TNF-a, IL-1a, IL-6, and IL-8 in whole saliva for the malignant transformation of oral lichen planus. Mol Carcinog. 2005;44:77–82.
55. Neppelberg E, Johannessen AC. DNA content, Cyclooxygenase-2 expression and loss of E-cadherin expression do not predict risk of malignant transformation in oral lichen planus. Eur Arch Otorhinolaryngol. 2007;264:1223–30.
56. Lopes MA, Feio P, Santos-Silva AR, Vargas PA. Proliferative verrucous leukoplakia may initially mimic lichenoid reactions. World J Clin Cases. 2015;3:861–3.
57. Bombeccari GP, Guzzi G, Tettamanti M, Giannì AB, Baj A, Pallotti F, Spadari F. Oral lichen planus and malignant transformation: a longitudinal cohort study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112:328–34.

58. González-Moles MA, Bascones-Ilundain C, Montoya JAG, Ruiz-Avila I, Delgado-Rodríguez M, Bascones-Martínez A. Cell cycle regulating mechanisms in oral lichen planus: molecular bases in epithelium predisposed to malignant transformation. Arch Oral Biol. 2006;51:1093–103.
59. Liu W, Shen ZY, Wang LJ, Hu YH, Shen XM, Zhou ZT, et al. Malignant potential of oral and labial chronic discoid lupus erythematosus: a clinicopathological study of 87 cases. Histopathology. 2011;59:292–8.
60. Gonzalez-Moles MA, Scully C, Gil-Montoya JA. Oral lichen planus: controversies surrounding malignant transformation. Oral Dis. 2008;14:229–43.
61. Krutchkoff DJ, Cutler L, Laskowski S. Oral lichen planus: the evidence regarding potential malignant transformation. J Oral Pathol. 1978;7:1–7.
62. van der Meij EH, Schepman KP, Smeele LE, van der Wal JE, van der Bezemer PD, Waal I. A review of the recent literature regarding malignant transformation of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;88:307–10.
63. Mignogna MD, Fedele S, Lo Russo L. Dysplasia/neoplasia surveillance in oral lichen planus patients: a description of clinical criteria adopted at a single centre and their impact on prognosis. Oral Oncol. 2006;42:819–24.
64. Otero-Rey EM, Suarez-Alen F, Peñamaria-Mallon M, Lopez-Lopez J, Blanco-Carrion A. Malignant transformation of oral lichen planus by a chronic inflammatory process. Use of topical corticosteroids to prevent this progression? Acta Odontol Scand. 2014;72:570–7.
65. Alves MGO, Balducci I, Carvalho YR, Cabral LAG, Nunes FD, Almeida JD. Evaluation of the expression of p53, MDM2, and SUMO-1 in oral lichen planus. Oral Dis. 2013;19:775–80.
66. Poomsawat S, Buajeeb W, Khovidhunkit SP, Punyasingh J. Overexpression of cdk4 and p16 in oral lichen planus supports the concept of premalignancy. J Oral Pathol Med. 2011;40:294–9.
67. Zhou G, Xia K, Du GF, Chen XM, Xu XY, Lu R, et al. Activation of nuclear factor-kappa B correlates with tumor necrosis factor-alpha in oral lichen planus: a clinicopathologic study in atrophic-erosive and reticular form. J Oral Pathol Med. 2009;38:559–64.
68. Santoro A, Maiorana A, Bardellini E, Festa S, Sapelli P, Facchetti F. NF-kappaB expression in oral and cutaneous lichen planus. J Pathol. 2003;201:466–72.
69. Segura S, Rozas-Muñoz E, Toll A, Martın-Ezquerra G, Masferrer E, Espinet B, et al. Evaluation of MYC status in oral lichen planus in patients with progression to oral squamous cell carcinoma. Br J Dermatol. 2013;169:106–14.
70. Xu Z, Shen Z, Shi L, Sun H, Liu W, Zhou Z. Aldehyde dehydrogenase 1 expression correlated with malignant potential of oral lichen planus. Ann Diagn Pathol. 2013;17:408–11.
71. Santos-Silva AR, Ribeiro AC, Soubhia AM, Miyahara GI, Carlos R, Speight PM, et al. High incidences of DNA ploidy abnormalities in tongue squamous cell carcinoma of young patients: an international collaborative study. Histopathology. 2011;58:1127–35.
72. Gouvêa AF, Santos Silva AR, Speight PM, Hunter K, Carlos R, Vargas PA, et al. High incidence of DNA ploidy abnormalities and increased Mcm2 expression may predict malignant change in oral proliferative verrucous leukoplakia. Histopathology. 2013;62:551–62.
73. Pentenero M, Monticone M, Marino R, Aiello C, Marchitto G, Malacarne D, et al. High-resolution DNA content analysis of microbiopsy samples in oral lichen planus. Oral Dis. 2017;23:318.
74. Acha-Sagredo A, Jiménez Y, Bagán JV, Echebarria-Goicouria MA, Aguirre-Urizar JM. Cytometric analysis of oral scrapings of patients with oral lichen planus. Cytopathology. 2011;22:106–10.
75. Maraki D, Yalcinkaya S, Pomjanski N, Megahed M, Boecking A, Becker J. Cytologic and DNA-cytometric examination of oral lesions in lichen planus. J Oral Pathol Med. 2006;35:227–32.
76. Sperandio M, Klinikowski MF, Brown AL, Shirlaw PJ, Challacombe SJ, Morgan PR, et al. Image-based DNA ploidy analysis aids prediction of malignant transformation in oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;21:643–50.
77. Hosni ES, Yurgel LS, Silva VD. DNA ploidy in oral lichen planus, determined by image cytometry. J Oral Pathol Med. 2010;39:206–11.

78. Accurso BT, Warner BM, Knobloch TJ, Weghorst CM, Shumway BS, Allen CM, et al. Allelic imbalance in oral lichen planus and assessment of its classification as a premalignant condition. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112:359–66.
79. Zhang L, Michelsen C, Cheng X, Zeng T, Priddy R, Rosin MP. Molecular analysis of oral lichen planus. A premalignant lesion? Am J Pathol. 1997;151:323–7.
80. Nylander E, Ebrahimi M, Wahlin YB, Boldrup L, Nylander K. Changes in miRNA expression in sera and correlation to duration of disease in patients with multifocal mucosal lichen planus. J Oral Pathol Med. 2012;41:86–9.
81. Danielsson K, Wahlin YB, Gu X, Boldrup L, Nylander K. Altered expression of miR-21, miR-125b, and miR-203 indicates a role for these microRNAs in oral lichen planus. J Oral Pathol Med. 2012;41:90–5.
82. Dang J, Bian YQ, Sun JY, Chen F, Dong GY, Liu Q, et al. MicroRNA-137 promoter methylation in oral lichen planus and oral squamous cell carcinoma. J Oral Pathol Med. 2013;42:315–21.
83. Lanfranchi HE, Aguas SC, Sano SM. Malignant transformation of atypical oral lichen planus: a review of 32 cases. Med Oral. 2003;8:2–9.
84. Markopoulos AK, Antoniades D, Papanayotou P, Trigonidis G. Malignant potential of oral lichen planus; a follow-up study of 326 patients. Oral Oncol. 1997;33:263–9.
85. Salem G. Oral lichen planus among 4277 patients from Gizan, Saudi Arabia. Community Dent Oral Epidemiol. 1989;17:322–4.
86. Silverman S Jr, Gorsky M, Lozada-Nur F, Giannotti K. A prospective study of findings and management in 214 patients with oral lichen planus. Oral Surg Oral Med Oral Pathol. 1991;72:665–70.
87. Silverman SJ, Gorsky M, Lozada-Nur F. A prospective follow-up study of 570 patients with oral lichen planus: persistence, remission, and malignant association. Oral Surg Oral Med Oral Pathol. 1985;60:30–4.
88. Torrente-Castells E, Figueiredo R, Berini-Aytés L, Gay-Escoda C. Clinical features of oral lichen planus. A retrospective study of 65 cases. Med Oral Patol Oral Cir Bucal. 2010;15:e685–90.
89. Sigurgeirsson B, Lindelöf B. Lichen planus and malignancy. An epidemiologic study of 2071 patients and a review of the literature. Arch Dermatol. 1991;127:1684–8.

Chapter 7
Systemic Diseases with an Increased Risk of Oral Squamous Cell Carcinoma

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Introduction

There is a growing awareness of the important relationships between systemic inflammatory diseases, infections, genetic disorders, medical therapies and cancer risk. A number of systemic disorders have been associated with an increased risk of head and neck squamous cell carcinoma. These include autoimmune conditions, genetic syndromes, infections, iatrogenic causes such as haematopoietic stem cell transplantation and graft-versus-host disease and rare associations including novel drugs. This chapter will discuss conditions which confer an increased risk of head and neck squamous cell carcinoma, as well as review the evidence for diseases which have been historically associated with oral cancer.

Autoimmune Polyendocrinopathies

Introduction

The autoimmune polyendocrinopathy syndromes are a heterogeneous group of disorders, characterised by the development of multiple autoimmune phenomena. This group consists of autoimmune polyendocrine syndrome type 1 (APS-1) and type 2 (APS-2) and x-linked polyendocrinopathy, immune dysfunction and diarrhoea

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(XPID) syndrome [1]. APS-1 is also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and has a known association with oral squamous cell carcinoma.

APS-1 (OMIM 240300) is a rare autosomal recessive syndrome caused by mutations in an autoimmune suppressor gene known as *AIRE*. This gene encodes a transcription factor and is involved in the generation of self-tolerance [1]. The classic triad of conditions present in this disorder includes mucocutaneous candidiasis, hypoparathyroidism and adrenocortical insufficiency, and the presence of two of these conditions is required for diagnosis [2].

Epidemiology

APS-1 is a rare syndrome and is most prevalent in the Finnish, Iranian, Jewish and Sardinian populations [3]. The majority of presentations occur under the age of 18 years, with mucocutaneous candidiasis the initial presenting feature in 60% of cases, followed by the development of hypoparathyroidism and adrenocortical insufficiency [2]. Italian and Finnish case series have reported the median age for initial diagnosis of mucocutaneous candidiasis as 6.5 years and 5.4 years, respectively [2, 4]. However, a large Finnish series reported that some patients did not develop the diagnostic dyad until adulthood [2].

Pathophysiology

The syndrome is caused by mutations in the *AIRE* gene, which is involved in the generation of self-tolerance. However the clinical presentation is diverse, so it is considered likely that the pathophysiology is more complex than abnormalities in this one gene [5]. *AIRE* has an important role in the elimination of autoreactive thymocytes during the development of the immune system [5]. If this process fails, then autoreactive T-lymphocytes will be released into the circulation, potentially causing end-organ damage and accompanying autoimmune disease.

Chronic mucocutaneous candidiasis (CMC) is the most commonly observed feature of APS-1, and it is suggested that a T-cell defect with a resultant poorly developed response to *C. albicans* may be the cause of this opportunistic infection [6]. APS-1 patients mount a satisfactory humoral immune response to *Candida* species and thus are not at risk of systemic candidiasis [5]. Further research into the mechanisms of this specific immunodeficiency have shown the presence of autoantibodies to IL-17F, IL-17A and IL-22 in APS-1 patients [7, 8]. These are key cytokines involved in defence against mucocutaneous candidal infection. Thus it appears that the increased susceptibility to mucocutaneous candidal infection in these patients is also a manifestation of autoimmunity.

Clinical Features

In addition to the classic triad of chronic mucocutaneous candidiasis (CMC), hypoparathyroidism and adrenocortical insufficiency, the clinical presentation can include a variety of less common conditions, including insulin-dependent diabetes mellitus, hypothyroidism, hypogonadism, pernicious anaemia, hepatitis, diarrhoea, keratitis, alopecia, vitiligo and enamel hypoplasia [9]. The most common initial manifestation of the condition is CMC, which may present in infancy and early childhood. Candidiasis may affect the skin, nails, oesophagus and oral and vaginal mucosa [4]. Oral candidiasis persisting beyond the newborn period and in association with any form of endocrinopathy or the rarer components of the syndrome (e.g. keratoconjunctivitis or vitiligo) should alert the clinician to the possibility of APS-1 [2, 3].

Oral and oesophageal candidiasis in APS-1 patients may present as mild intermittent angular cheilitis but may also cause significant disease. Clinical manifestations include erosive, hyperplastic and atrophic forms of candidiasis, leukoplakia-like areas of the oral mucosa and oesophageal webbing and strictures [9]. CMC is also the most common manifestation of APS-1 and is present in all adult patients with the syndrome [2]. The majority of APS-1 patients are colonised by *C. albicans* [6].

Symptoms related to the endocrine components of the syndrome may be subtle initially, and a high index of clinical suspicion is required. Symptoms of hypocalcaemia may be non-specific, such as muscle cramping during infections and mild paraesthesia or clumsiness [3]. Adrenal insufficiency presents with extreme fatigue, pigmentation, weight loss and hypotension. These conditions are potentially life-threatening, so identification of patients with unusual or chronic oral mucosal candidal infection could be the key to prompt investigation and recognition of potentially serious endocrinopathies.

Oral Cancer Risk

The largest case series of APS-1 published to date, involving 92 Finnish patients, has demonstrated that the prevalence of oral and oesophageal squamous cell carcinoma (SCC) in this patient group is up to 10% in patients over the age of 25 years [6]. The patients diagnosed with oral or oesophageal SCC in this series were aged between 29 and 44 years. In the general population, the mean age at diagnosis for oral and oesophageal SCC is greater than 60 years, and it is considered an uncommon disease in younger people. The literature contains several case reports regarding young patients developing multiple oral SCCs in the context of APS-1 [10, 11].

The role of chronic candidal infection in the aetiology of oral and oesophageal SCC has been a topic of considerable debate. Historical animal studies have demonstrated the generation of carcinogenic chemicals such as nitrobenzylmethylamine by *C. albicans*, with an associated increase in the incidence of oral SCC [12]. A study conducted in Finland utilising *C. albicans* species isolated from APS-1 patients found that these organisms could synthesise potentially carcinogenic levels

of acetaldehyde from glucose and alcohol [13]. Experts in the field consider that given the long duration of candidal infection in APS-1 patients and the tendency for oral and oesophageal SCCs to develop in sites of chronic candidal infection, this infection is an important factor in the development of the malignancy. However the extent of this role is as yet unknown. Given that the syndrome features a number of phenomena related to abnormal T-cell function and that these cells have a key role in antitumour effects, it is likely that the relationship to the development of malignancy is more complex than simply chronic candidal infection.

Given the high rate of oral and oesophageal SCC in young people with APS-1 and the potential contribution of chronic mucosal candidal infection to carcinogenesis, it is important that appropriate management and screening are undertaken in this population. APS-1 patients should have regular reviews by a multidisciplinary team comprising oral medicine specialists, otolaryngology/head and neck surgery and endocrinology specialists. Careful attention to screening of the oral and oesophageal mucosa should be maintained, and any suspicious lesions should be biopsied. It is recommended that treatment of oral and oesophageal candidiasis should be aggressive [2–4, 6]. This may be best achieved with a combination of topical and systemic therapy, and careful attention should be paid to monitoring for resistant strains of *C. albicans*, as this is a concern with the potential long-term use of azole antifungals in this patient cohort [14]. It is recommended that the use of azole antifungals is limited to two to three courses per year in order to reduce the risk of generation of resistance [3]. It is essential that patients with APS-1 are educated regarding the risk of oral and oesophageal cancer and that they clearly understand the index symptoms and self-monitoring procedures and know when to alert a clinician. They should also be counselled regarding avoidance of other known risk factors for oral and oesophageal SCC such as smoking, alcohol and betel quid use.

Lupus Erythematosus

Introduction

Lupus erythematosus (LE) is a group of diverse persistent autoimmune inflammatory diseases which frequently affect the skin and oral mucosa. Discoid lupus erythematosus (DLE) is the most common chronic form of cutaneous lupus. It is characterised by persistent scaly, disc-like plaques on the scalp, face and ears and may cause pigmentary changes, scarring and hair loss. The oral mucosa may be involved.

Epidemiology

The prevalence of DLE has been reported to be less than five in 10,000 individuals [15]. The most common age of onset of DLE is between 20 and 40 years [16]. It affects both females and males, with a slight female predominance. The female-to-male ratio

has been reported between 3:2 and 2:1 compared with 12:1 in systemic lupus erythematosus (SLE) [17]. Unlike SLE, there does not seem to be any racial predisposition to DLE. However, reports in the United States suggest that DLE may be slightly more common in Black Americans than in White Americans [18]. Smoking has been linked to both the development and severity of DLE [19].

Clinical Presentation

The typical cutaneous manifestations of DLE are white keratinised plaques with elevated borders, central atrophy, radiating white striae and telangiectasia. It has a prolonged clinical course and can persist for many years despite various treatments. Oral manifestations are present in up to 25% of patients and are located most commonly on the buccal mucosa, followed by the gingivae, labial mucosa and vermillion of the lip [20]. Oral mucosal lesions have an irregular white border with telangiectasia, surrounding a central atrophic area in which there may be small white papules. It may also present as irregular erythematous areas without a keratotic component. Oral manifestations may be present without cutaneous lesions. DLE is more likely to affect the palatal mucosa than lichen planus, and the typical appearance of an erythematous area with radiating white striae can sometimes differentiate the lesion clinically from lichen planus and lichenoid reactions to metal restorations (typically amalgam) or medications [21]. DLE lesions frequently appear on the lower lip, due to the photosensitive nature of this condition.

Pathophysiology

DLE is an autoimmune disease; however the precise pathophysiology of the disorder is unclear. It is suggested that UV light-induced epidermal inflammation, autoantigen localisation and basal keratinocyte apoptosis contribute to the process. However, it is likely that multiple components of the innate and adaptive immune system are involved in the pathogenesis of this condition [22].

Management

If symptomatic, the oral manifestations of DLE are managed in a similar manner to other oral mucosal inflammatory conditions. This is primarily with topical corticosteroids and immunomodulators, although on occasion, systemic immunosuppression may be required, particularly for refractory lower lip lesions.

Oral lesions in DLE need close clinical monitoring, given the possibility of developing dysplasia or malignancy in these sites. In order to rule out the presence of dysplastic change, incisional biopsy may be required, in some cases serially as

part of active clinical monitoring. UV-B light exposure can precipitate DLE lesions and increase the risk of sun-related malignancy; therefore it is important to examine the lips carefully and give sun protection advice.

Oral Cancer Risk

The evidence regarding oral carcinoma development in sites affected by oral DLE lesions is limited to a number of case reports. The risk of developing malignant change in oral mucosal DLE is uncertain [21]. DLE-related malignant transformation affecting the lip has been reported, particularly in males. However this is confounded by the fact that this is a sun-exposed site and the excess risk may be due to ultraviolet radiation rather than the underlying inflammatory disorder [23].

Fanconi Anaemia

Introduction

Fanconi anaemia (FA) is a rare disorder with a heterogeneous clinical picture. It is primarily an autosomal recessive inherited condition [24]. The condition is caused by mutations in one of at least 21 genes known to be involved in the pathogenesis of the condition [25]. These genes are involved in DNA repair, and cardinal features of FA are genomic instability and extreme sensitivity to DNA cross-linking agents. Clinically the condition is characterised by variably expressed congenital abnormalities, early bone marrow failure, and an increased risk of cancer, particularly acute myeloid leukaemia and squamous cell carcinomas of the head and neck and anogenital regions [26].

Epidemiology

FA is found in all races and ethnic groups and has a worldwide prevalence of one to five per million. It is slightly more common in males. Average life expectancy is 20 years [26]. Given the heterogeneity of clinical presentations, the age of diagnosis can be extremely variable. The majority of patients will develop haematological abnormalities, and the median age of onset of these is 7 years [27]. Patients with a more severe phenotype tend to develop bone marrow failure and haematological malignancy at an earlier age. However a cohort of patients with mild manifestations of FA have survived into early adulthood, and the development of solid tumours such as head and neck SCC at a young age has been the first indication of the presence of the condition [28]. In addition, the use of haematopoietic stem cell

transplantation has enabled the survival of patients with more severe manifestations of FA, and the development of solid tumours in these longer-term survivors with FA has become a significant issue [27].

Researchers comment that it is possible that the condition is more common than previously thought, due to under-recognition of the variety of clinical manifestations which are possible in the disorder [29].

Pathophysiology

The majority of cases of FA are autosomal recessive, with the exception of FANCB which is x-linked [30]. The disorder results from biallelic inactivation of one of at least 21 genes which have been identified as related to the condition [25]. The proteins encoded by genes involved in FA are essential for the repair of DNA interstrand cross-links.

Due to its critical role in DNA repair, FA is considered a "caretaker gene disease" and is grouped with other similar conditions including ataxia telangiectasia, Bloom syndrome, hereditary non-polyposis colorectal cancer and hereditary breast/ovarian cancer syndromes [26].

Research into the molecular genetics of FA has established significant interactions between the FA and BRCA pathways, which are associated with hereditary forms of breast and ovarian cancer. It has been confirmed that two of the FA genes are BRCA1 and BRCA2 [25]. The DNA repair pathway mediated by these genes and their protein products is now known as the FA-BRCA pathway, and further research is ongoing into the mechanisms of increased cancer risk related to these genetic abnormalities.

The mechanisms by which the genetic abnormalities cause disease manifestations in FA are gradually being elucidated. It is suggested that the genomic instability resulting from ineffective repair of DNA damage results in increased cancer development [31]. The mechanisms by which other disease manifestations occur are less clear; however it is thought likely that the FA proteins participate in other ways to preserve genomic integrity and that deficiencies in these processes lead to susceptibility to other diseases and abnormalities [27, 32].

Clinical Features

Clinical manifestations of FA range from very severe congenital abnormalities to mild clinical features. The major features of the condition are congenital malformations (short stature, radial-ray anomalies, café au lait spots, cardiac and renal anomalies), early bone marrow failure, hypersensitivity to DNA cross-linking agents and an increased risk of haematological and solid malignancy. Multiple other congenital anomalies have been reported in association with FA; however up to 40% of patients

have no major physical malformations [30]. Comprehensive reviews exist on the anomalies associated with this disorder [33]. Given the wide phenotypic variation, it is important that FA is considered in any child or young adult diagnosed with aplastic anaemia, myelodysplastic syndrome, acute myeloid leukaemia or squamous cell carcinoma of the head and neck region [30, 34]. FA should also be considered in a young person who has an abnormally severe adverse response to chemotherapy or radiotherapy [29]. A family history of multiple cancers and/or excessive toxicity from radiotherapy or chemotherapy should trigger the clinician to consider FA [25].

Diagnosis of FA is based on a combination of observed clinical features and laboratory testing, which consists of demonstrating hypersensitivity to DNA cross-linking agents by exposing cells from the peripheral blood, bone marrow or skin to diepoxybutane or mitomycin C [33]. Cells from patients with FA will exhibit increased rates of chromosomal breakage when exposed to such agents. Additional genetic testing is performed to identify the specific mutation present and assign the patient to a complementation group [30]. Some correlation between genotype and phenotype has been established, with a more severe phenotype associated with complementation groups FANCD1 and FANCN. However there are few other strict associations between specific complementation groups and their clinical manifestations [30].

Oral Cancer Risk

One of the most significant features of FA is the dramatically increased risk of SCC of the head and neck region (HNSCC). Studies based on case series and large FA registries have estimated the risk as 500–700 times higher than the general population [31, 35].

Previously, the survival rate for patients with FA was poor due to early-onset bone marrow failure and haematological malignancy. However with the improvements in therapy for this condition, particularly the use of haematopoietic stem cell transplantation (HSCT), patients are surviving longer, and the high incidence of solid tumours in this population is becoming evident. Life expectancy with FA is still relatively short (median 20 years), and it is possible that studies still underestimate the risk of solid tumour development due to competing causes of mortality [27, 36]. Patients with a milder disease phenotype may survive to young adulthood without the development of haematological abnormalities, and unusually early presentation with a solid tumour such as HNSCC may be the first manifestation of FA in these patients [28, 37].

HNSCCs are the second most common cancer in FA patients, and the cumulative incidence of developing HNSCC is 14% by 40 years of age [35]. FA patients develop HNSCC at a very young age compared to the general population, with a mean age of 32 years at development of first HNSCC [28]. Case reports of the development of HNSCC in FA patients as young as 13 years exist [38].

The role of traditional risk factors for HNSCC in FA patients is not as significant as in the general population, with only a quarter of patients in a large cohort study having been exposed to alcohol or tobacco prior to the development of HNSCC [28]. This is in contrast to the general population, where at least 75–85% of HNSCC may be attributable to a history of tobacco and alcohol exposure [39].

In the same cohort study, which represents the largest case series of HNSCC in FA patients ($n = 35$), the most common site of HNSCC in FA patients was the oral cavity, particularly the tongue, in concordance with previous studies [28]. In this series two patients developed a second HNSCC, and half of the patients developed a second tumour (anal, cervical, vulvar or cutaneous SCC). Half of the patients experienced recurrence of their tumour [28].

Survival rates for FA patients with HNSCC are significantly poorer than the general population. In one cohort study, 64% of the patients died as a result of HNSCC. Five-year cause-specific survival rates were only 47% in this group [28]. In contrast, survival rates in other studies for patients under 35 years of age and treated for HNSCC are >55% [40, 41]. It has been suggested that the genomic instability in FA leads to the development of more aggressive tumour types and early recurrence [28].

Due to FA-mediated sensitivity to radiation and chemotherapy, the mainstay of treatment of HNSCC in this population is surgery. Studies have shown that FA patients can tolerate standard surgical procedures for HNSCC, including free flap reconstruction [28]. If haematological abnormalities are present, then these may present difficulties perioperatively and require the involvement of a haematologist [28]. Radiation-induced pancytopenia is a rare complication in the general population but has been observed significantly more frequently in FA patients, and complications such as severe mucositis are more common [28]. Radiotherapy can be used for management of HNSCC in FA patients; however close monitoring is required to detect toxicity, and dosage reductions may be required [42]. FA patients are extremely sensitive to toxicity from chemotherapeutic agents commonly used as adjunct treatment modalities for HNSCC, particularly cisplatin-based chemotherapy regimens, as these drugs are cross-linking agents. These factors mandate modifications of adjunct chemotherapy and radiotherapy regimens in this patient population [28, 32, 43]. Ideally, management of HNSCC in FA patients should be surgical, necessitating good screening programmes in order to detect tumours early, when they are amenable to primary surgical resection [34, 43].

Recommendations on screening for FA patients advise that examination of the oral cavity and oropharynx should begin at 10–12 years of age, particularly if the patient has undergone HSCT or has any oral mucosal manifestations of GVHD or leukoplakia. Patients with abnormal oral mucosa will require six to eight weekly examinations, and those with normal mucosal appearance should be examined at least three monthly [28, 34, 35, 44]. A low threshold for biopsy of oral mucosal abnormalities should be maintained, as the aim of screening is to detect HNSCC when the tumours are small and amenable to primary resection.

An additional consideration for clinicians involved in the diagnosis and management of HNSCC is that in up to 20% of patients with FA described in the literature,

the development of HNSCC at a young age is the first presenting feature of undiagnosed FA [37]. These patients had only mild physical manifestations of FA and few or no haematological abnormalities. It is important to maintain a high index of suspicion and refer appropriately for screening for FA in young patients with HNSCC. It is particularly important that these patients are identified prior to commencement of therapy, given the high level of toxicity which will be experienced by FA patients treated with standard HNSCC treatment protocols including radiotherapy and chemotherapy with cross-linking agents [37].

Two major areas of controversy in the literature are regarding the nature of the relationship between HNSCC risk in FA patients and their exposure to human papilloma virus (HPV) and HSCT and/or graft-versus-host disease (GVHD).

High-risk HPV-related SCC of the oropharynx is increasing in incidence in the general population and more particularly in young people diagnosed with HNSCC [45]. When considering the high risk of HNSCC in patients with FA, researchers have suggested that the immunodeficiency state associated with FA leads to increased mucosal susceptibility to infection with viruses such as HPV and also increased sensitivity to known carcinogens such as alcohol and tobacco smoking [35]. The basis of the theory regarding HPV as a significant factor in the aetiology of HNSCC in FA patients is that the HPV16 E7 oncoprotein has been demonstrated to interact with the FA pathway and that genes involved in the FA pathway are critical for the repair of DNA damage induced by E7 [46, 47]. Additionally, case series from North America have demonstrated a high rate of high-risk HPV DNA in HNSCCs from FA patients [48]. In stark contrast, however, examination of HNSCC cases from FA patients in Europe failed to demonstrate this link, and in fact the majority of tumours were negative for HPV DNA [49]. In view of this conflict within the literature, it is difficult to establish the true nature of the relationship between high-risk HPV and HNSCC in FA patients. However many centres recommend HPV vaccination for all FA patients in the hope that it may assist in preventing some of these malignancies [28, 31, 32, 34, 46].

The use of haematopoietic stem cell transplantation (HSCT) has led to significantly improved survival for patients with FA. The increased longevity conferred by HSCT has allowed other features of the cancer susceptibility of FA to emerge, leading to the observation of a dramatically increased risk of HNSCC and other epithelial cancers in patients with FA [34]. In FA patients, the extreme sensitivity to chemotherapy and radiotherapy has led to alterations in HSCT protocols to reduce transplant-related mortality [30]. A cohort study comparing the risk of SCC and SCC-related mortality between two groups of FA patients showed that the rate of SCC development in transplanted patients was 4.4 times higher than those who did not receive transplants [31]. This relative risk is of similar order to non-FA patients receiving HSCT; however the increased risk on top of the already high baseline FA risk of HNSCC leads to a very significant overall increased risk of HNSCC in these patients [50]. Transplanted patients also developed HNSCC at an earlier age than non-transplanted patients (median 18 years vs. 33 years). This study also demonstrated that acute and chronic GVHD were significant risk factors for development of HNSCC in this population [31]. The study found that survival and outcomes fol-

lowing diagnosis of HNSCC were poor in both cohorts, with a median survival of 13 months [31]. A study of 13 FA patients with HNSCC following HSCT showed a median survival time of 6 months [44].

In summary, FA is a genetic disorder with a greatly increased risk of malignancy, particularly HNSCC. Clinicians should maintain a high index of suspicion for this condition in young people diagnosed with HNSCC. Treatment regimens require alteration in this patient population due to increased toxicity in response to chemotherapy and radiotherapy. Very intensive clinical monitoring and surveillance regimes are essential for all FA patients, in order to detect HNSCC whilst still amenable to primary surgical resection.

Dyskeratosis Congenita

Introduction

Dyskeratosis congenita (DC), an inherited disorder of telomere function, is characterised by a classic clinical triad of dysplastic nails, lacy reticular pigmentation of the upper chest and/or neck and oral leukoplakia. Individuals with this disorder have an increased risk of bone marrow failure, pulmonary fibrosis and other cancers. The condition may be x-linked, autosomal dominant or autosomal recessive [51].

Epidemiology

The prevalence of DC in the general population is not known, and it is believed to be rare. As of 2015, one reviewer was aware of at least 400 families in the world [52].

Pathophysiology

The mode of inheritance of DC varies by the affected genes:

- X-linked: *DKC1*
- Autosomal dominant: *TERC* and *TINF2*
- Autosomal dominant or autosomal recessive: *ACD*, *RTEL1* and *TERT*
- Autosomal recessive: *CTC1*, *NHP2*, *NOP10*, *PARN* and *WRAP53*

The link between DC and cancer is ascribed to the finding that patients with DC have defects in telomere maintenance. Telomeres are complex DNA-protein structures that protect chromosomal ends from degradation and inappropriate recombination [53].

Patients with DC have very short telomeres, and mutations have been identified in telomere biology genes, thus predisposing these patients to carcinogenesis. To date there has been no comprehensive quantitative analysis of cancer risk in DC [54].

Clinical Presentation

Dyskeratosis congenita is characterised by a classic clinical triad of dystrophy of the nails, lacy reticular cutaneous pigmentation and oral leukoplakia. Mucosal leukoplakias are seen in approximately 80% of patients and are most frequently present on the oral mucosa. Areas of leukoplakia typically involve the lingual mucosa, buccal mucosa, palate and most commonly tongue. The leukoplakia may become verrucous, and ulceration may occur. Patients also may have an increased prevalence and severity of periodontal disease, dental caries, hypoplastic enamel and hypodontia [55].

Patients with DC are at increased risk for progressive bone marrow failure (BMF), myelodysplastic syndrome (MDS) or acute myelogenous leukaemia (AML), solid tumours (usually squamous cell carcinoma of the head/neck or anogenital cancer) and pulmonary fibrosis. Other findings can include abnormal pigmentation changes not restricted to the upper chest and neck, eye abnormalities (epiphora, blepharitis, sparse eyelashes, ectropion, entropion, trichiasis) and dental abnormalities (caries, periodontal disease, taurodontism). Although most patients with DC have normal psychomotor development and normal neurologic function, significant developmental delay is present in the two variants which include cerebellar hypoplasia (Hoyeraal-Hreidarsson syndrome) and bilateral exudative retinopathy and intracranial calcifications (Revesz syndrome). The onset and progression of manifestations of DC vary—at the mild end of the spectrum are those who have only minimal clinical signs with normal bone marrow function and at the severe end are those who have the complete diagnostic triad and early-onset bone marrow failure [52].

Cancer in patients with DC usually occurs in the third decade with head and neck cancer and MDS being the most common malignancies. In older patients MDS/AML is more prevalent. Patients with moderate or mild forms of DC or those who have received haematopoietic stem cell transplant for bone marrow failure related to DC are more likely to develop solid tumours, whereas patients with severe forms of DC usually die from the disease before the development of malignancy.

Management

Treatment is tailored to the individual. Haematopoietic cell transplantation (see previous section) is the only curative treatment for bone marrow failure and leukaemia but historically has had poor long-term efficacy. If a suitable donor is not available, androgen therapy may be considered for bone marrow failure. Treatment of other cancers is tailored to the type of cancer. Cancer therapy may pose an increased risk

for prolonged cytopenias as well as pulmonary and hepatic toxicity, due to the underlying manifestations of DC. Treatment of pulmonary fibrosis is primarily supportive, although lung transplantation may be considered [51, 54, 56].

Genetic counselling regarding the risk to family members depends on accurate diagnosis, determination of the mode of inheritance in each family and results of molecular genetic testing. Once the DC-related pathogenic variant(s) have been identified in an affected family member, prenatal testing for a pregnancy at an increased risk for DC is possible [52].

Oral Cancer Risk

Data from the United Kingdom Dyskeratosis Registry indicated that the crude rate of malignancy amongst approximately 300 patients was 10% [56].

The quantitative risk of developing any cancer in DC is approximately 50-fold that of the general population, and the risks of specific malignancies are very high, in the thousands-fold for squamous cell carcinomas. The most frequent solid tumour in DC is head and neck squamous cell carcinoma, comprising 40% of all cancers found in these patients [54]. Head and neck cancer risks and outcomes in this population are similar to those encountered in Fanconi anaemia, as discussed above.

Palmoplantar hyperkeratosis

Introduction

Palmoplantar hyperkeratosis is a group of hereditary disorders of keratinisation involving hyperkeratosis of the palms and soles.

Epidemiology

The prevalence of the disorder in the general population is unknown but is likely to be less than 1 in 1,000,000. It usually manifests clinically at a very young age [57].

Pathophysiology

Two different forms of palmoplantar hyperkeratosis have been mapped to the type 1 and type 2 keratin gene cluster on chromosome 17, although palmoplantar hyperkeratosis has been shown to be heterogeneous clinically and genetically. The disorder may be caused by mutations in keratins as well as nonkeratins [58].

Clinical Presentation

Clinically and histopathologically different forms of palmoplantar hyperkeratosis exist. These may be distinguished by the pattern of the palmar and plantar keratoses, the age of onset of the disease and the occurrence of any associated features. The combination of palmoplantar keratosis with oral hyperkeratosis was first described by Fred et al. in 1964, and subsequent case reports of hyperkeratosis affecting the palms, soles and oral mucosa exist [59]. Oral manifestations have been described as presenting on the labial attached gingivae and areas of the oral mucosa affected by friction and irritation. The oral hyperkeratosis often has a symmetrical distribution and appears in early childhood or puberty, and the lesions increase in severity with age. Subungual and circumungual hyperkeratosis maybe an associated feature [60]. There is a strong association with oesophageal carcinoma in these patients [61].

Management

Management is focused on surveillance for early detection and treatment of oesophageal dysplasia. Surveillance includes annual gastroscopy with biopsy of any suspicious lesion together with screening biopsies from the upper, middle and lower oesophagus. This is coupled with dietary and lifestyle modification advice and symptom education. Genetic counselling can be offered to patients and family members once a family history has been established [62].

Oral Cancer Risk

There are significant associations between palmoplantar hyperkeratosis and oesophageal cancer [58, 62]. Although the oral lesions are generally considered benign, case reports of oral cancer in patients with palmoplantar hyperkeratosis, which is likely to have occurred in areas of oral leukoplakia related to the condition, exist [63].

Xeroderma Pigmentosum

Introduction

Xeroderma pigmentosum (XP, OMIM 278700) is an autosomal recessive genetic disorder characterised by an inability to repair DNA damage caused by ultraviolet light exposure. Individuals with this disorder have an increased risk of developing

skin cancer, and about 50% of children with XP will develop a cutaneous malignancy by the age of 10 years. Most patients with XP develop multiple skin cancers (basal cell carcinoma, squamous cell carcinoma, melanoma) in sun-exposed areas such as the face, lips, eyes, eyelids and scalp [64, 65]. Review of the literature has revealed a number of case reports of oral cavity neoplasms in individuals with XP, particularly squamous cell carcinoma of the tip of the tongue, a presumed sun-exposed location [66]. An increased risk of other forms of cancer exists in this syndrome, and gliomas of the brain and spinal cord; tumours of the lung, uterus, breast, pancreas, stomach, kidney and testicles; and leukaemia have been reported in individuals with XP [67]. Neurological symptoms are also a feature of the syndrome.

Epidemiology

XP has variable prevalence, affecting one per million in the United States, 2.3 per million in Western Europe and 45 per million in Japan [68]. Affected populations have also been described in North Africa and the Middle East, with less clearly understood frequency.

Pathophysiology

The genes affected in XP are involved in the repair of DNA damage secondary to ultraviolet light exposure. The processes affected include the nucleotide excision repair and post-replication repair pathways. The result is an accumulation of damaged DNA, which can result in increased malignant potential and neurological problems. Direct damage to the DNA of neural cells can also occur, but the precise mechanisms of this are still unclear [68].

Clinical Presentation

XP predominantly affects the eyes and sun-exposed skin and usually presents in infancy or early childhood. Affected children can develop sunburn after spending just a few minutes in the sun, and the redness and blistering can last for weeks. The skin is dry and scaly with irregular pigmentation. Extreme ocular sensitivity to UV light is a characteristic feature, with resultant abnormalities of the conjunctiva, cornea, eyelashes and eyelids. Photophobia, keratitis and eyelid thinning are common features. Benign conjunctival growths are also a feature of the condition. Approximately one third of individuals with XP may develop neurological abnormalities including hearing loss, gait abnormalities, dysphagia, dysphonia and seizures [68].

Management

The mainstay of management involves avoiding exposure to sunlight. Regular periodic skin screening examinations and local management of skin cancers are required. Skin cancers may be managed surgically or medically with topical chemotherapeutic agents such as fluorouracil. Oral isotretinoin or acitretin may be used as a means of preventing the development of skin cancers but is teratogenic and has a number of other serious side effects. Regular ophthalmological screening is also required for the management of ocular complications [68].

Oral Cancer Risk

There is no published evidence quantifying the risk of oral cancer in patients with XP. A number of case reports have been published regarding oral squamous cell carcinomas in patients with XP, and the majority of these were on the tongue tip, which is arguably also a sun-exposed area [66].

Haematopoietic Stem Cell Transplantation and Graft-Versus-Host Disease

Introduction

Haematopoietic stem cell transplantation (HSCT) and bone marrow transplantation (BMT) have become the mainstay of treatment for a variety of diseases, including acute and chronic leukaemias, inherited immunodeficiencies and assorted haematological disorders. Successful transplantation can result in cure of many of these conditions. Improvements in transplant protocols and techniques have led to reductions in peri-transplant morbidity and mortality and prolonged survival following transplant, and the impact of transplant-related late effects has now become more evident. The prevention and management of transplant complications such as graft-versus-host disease (GVHD) and late second malignancies has become a significant focus of research. Studies on the development of solid tumours following HSCT have identified that there is a significantly increased risk of head and neck squamous cell carcinoma (HNSCC) in this patient group and that this is largely related to graft-versus-host disease. Graft-versus-host disease involves an immunological attack on the host by the transplanted immune cells from the donor. This section will consider the relationship between graft-versus-host disease and the risk of HNSCC.

Epidemiology

Acute graft-versus-host disease occurs in up to 80% of HSCT recipients [69, 70]. Risk factors for the development of acute GVHD include a HLA-mismatched donor, older donor age and the use of a female donor for a male recipient [70].

Chronic graft-versus-host disease (cGVHD) occurs in up to 80% of adult long-term survivors of HSCT [71]. Chronic GVHD is less common in children, occurring in up to 25% of children receiving HSCT [72]. The most significant risk factor for the development of chronic GVHD is a history of acute GVHD. Other risk factors for chronic GVHD include HLA-mismatched donors, older patient and donor ages, female donor to male recipient and the use of mobilised peripheral stem cells rather than bone marrow as the transplant source [70].

Early reports of solid tumour risk related to HSCT were confounded by the widespread use of total body irradiation (TBI) as part of the conditioning regime, leading to an increased risk of second malignancy. Over recent years, techniques of conditioning for HSCT have changed significantly, and it is likely that this will influence future outcomes regarding solid tumour risk. Increased understanding of the role of acute and chronic GVHD in the aetiology of second malignancies, particularly HNSCC, has led to changes in transplant techniques and GVHD prevention strategies.

Males appear to have a higher risk of post-HSCT solid tumour development, particularly squamous cell carcinoma (SCC) of the skin and oral cavity.

The risk of solid tumour development increases with time after transplant, with long-term survivors (>10 years) having a 25× increased risk of oropharyngeal cancer [73].

Studies have demonstrated that TBI used as part of transplant conditioning at a young age (younger than 10 years) leads to a greatly increased risk of development of a solid malignancy. However, this effect was limited to non-SCC solid tumours, and radiation-based conditioning did not lead to an increased risk of oropharyngeal SCC. Age at transplantation did not significantly affect the risk of GVHD-related oropharyngeal SCC [73].

Pathophysiology

Acute graft-versus-host disease develops in three distinct phases. Initially, tissue damage caused by conditioning regimens involving chemotherapy and radiotherapy leads to host tissue damage and the release of inflammatory cytokines. This results in increased expression of major histocompatibility complex (MHC) antigens on host antigen-presenting cells, allowing increased reactivity of donor cells against the host. Activated donor T-cells undergo clonal expansion and release

IL-2 and interferon gamma, leading to recruitment of cytotoxic T-cells and macrophages. A significant inflammatory response develops, which is further stimulated by molecules such as lipopolysaccharides which have been released from the damaged gut mucosa. The ongoing inflammation leads to further tissue damage and cytokine release, with amplification of the inflammatory response [74].

Chronic graft-versus-host disease is a "multisystem alloimmune and autoimmune-like disorder characterised by immune dysregulation, immune deficiency, impaired end-organ function and decreased survival" [75].

The fundamental pathophysiology of cGVHD relates to a situation of dysregulated and disordered immune reactivity against self and donor antigens [74]. The precise mechanisms of cGVHD are complex and likely to involve multiple elements of the donor and host immune systems. Pro-inflammatory T-cells play a major role; however regulatory T-cells and B-cells are also involved in the pathogenesis. Clinically and histologically cGVHD resembles a number of autoimmune diseases, including scleroderma, systemic lupus erythematosus, primary biliary cirrhosis, Sjogren syndrome and lichen planus [75].

Reduced intensity and non-myeloablative conditioning regimens were introduced with the aim of extending the availability of HSCT to patients who were deemed at high risk of complications related to traditional myeloablative pre-transplant conditioning [76]. Additionally, it was recognised that the "graft-versus-tumour" effect played a significant role in prevention of relapse. This refers to the ability of the donor T-cells to target and destroy any residual malignant cells in the host, such as may be the case after a non-myeloablative conditioning regime. The graft-versus-tumour effect has also been utilised in the development of protocols using donor lymphocyte infusions (DLI), in which a post-transplant patient is given infusions of immunocompetent lymphocytes from their original donor, to elicit a graft-versus-tumour effect with the aim of inducing remission [76]. However a significant graft-versus-tumour effect is often accompanied by active and sometimes severe graft-versus-host disease. Management of GVHD is a balance between high levels of immunosuppression to prevent it from occurring, which will lead to a reduction in the graft-versus-tumour effect and a higher risk of disease relapse, and the potential morbidity caused by severe GVHD.

Mechanisms of solid tumour development in post-HSCT patients or who have suffered from cGVHD are incompletely understood. Theories which have recently been suggested for the increased incidence of oral SCC in this patient cohort include the development of genomic instability in the mucosa following prolonged periods of inflammation and immunological "injury" by T-cells; long-term upregulation of cytokines which are known to be active in SCC, such as type 1 interferon; and possibly even a direct role of donor-derived cells in carcinogenesis [77–79].

Clinical Features

The classical clinical triad of acute GVHD is the involvement of the skin, gastrointestinal tract and liver. Skin manifestations include a maculopapular rash primarily affecting the palms and soles but which may affect any part of the body.

Gastrointestinal involvement is characterised by secretory diarrhoea which can be severe. Liver involvement manifests as jaundice and a cholestatic liver picture [69]. Oral mucosal involvement with acute GVHD is characterised by mucositis of varying severity [75].

Chronic GVHD has a variety of different features and organ involvement. The most commonly affected sites are the skin and oral mucosa. Oral mucosal manifestations of cGVHD include reticular white striae (or "lichen planus-like changes"), erythema, erosion and ulceration and hyperkeratotic plaques [80, 81]. Sclerotic cGVHD can lead to contraction of the skin around the mouth, causing microstomia, as well as fibrosis of the oral mucosa leading to trismus and limited flexibility of the oral mucosa. The major and minor salivary glands may also be affected by cGVHD, and this can lead to symptomatic xerostomia. Recurrent superficial mucoceles are also a feature [75].

Traditionally cGVHD was defined as occurring greater than 100 days post-transplant. However with the advent of reduced-intensity and non-myeloablative conditioning regimens, changes have been observed in the natural history of cGVHD. Some patients who have undergone reduced-intensity transplants will develop cGVHD very much later and may also develop signs of both acute and chronic GVHD following donor lymphocyte infusion (DLI) [82]. Hence the distinction between acute and chronic GVHD is now made based on clinical features rather than on time after transplantation. Various other presentations are also now recognised, such as "overlap" and "late acute" GVHD [80]. In an overlap presentation, features of acute and chronic GVHD are present simultaneously.

Diagnosis of cGVHD is made by recognising the presence of the "diagnostic" manifestations, as listed in the NIH criteria. The presence of a "diagnostic" sign or symptom establishes the presence of cGVHD without the need to perform further testing. The NIH criteria also list "distinctive" signs or symptoms, which are not sufficient to establish a diagnosis of cGVHD in isolation—further testing is required, such as histological diagnosis. A diagnosis of cGVHD is made if at least one diagnostic manifestation is present or at least one distinctive manifestation plus a positive diagnostic test, such as histopathological confirmation [80].

The only "diagnostic" manifestation of oral mucosal cGVHD is lichen planus-like changes. If this feature is present, then cGVHD may be diagnosed, without the requirement for a biopsy or further investigations. "Distinctive" manifestations include xerostomia, mucoceles, mucosal atrophy, ulcers and pseudomembranes [80]. Previous iterations of the diagnostic criteria included hyperkeratotic plaques without lichen planus-like features (leukoplakia) as a diagnostic criterion; however this has now been removed due to concerns that it represents a separate entity, with an associated risk of malignant transformation [80].

The major differential diagnoses for oral mucosal manifestations of cGVHD are inflammatory conditions such as oral lichen planus, from which it is clinically indistinguishable and infective processes such as oral candidiasis. If there is any doubt about the diagnosis, then consideration of the wider clinical picture is important, as is appropriate investigation of the oral mucosa to rule out infective or neoplastic processes.

Clinical manifestations which are considered "common" to both acute and chronic GVHD include pain, erythema, mucositis and gingivitis. The presence of these features is considered either diagnostic or distinctive for either type of GVHD [80].

Chronic GVHD can affect the skin, nails, genital mucosa, fascia, eyes, lungs and the gastrointestinal tract [83].

A variety of strategies have been employed in order to prevent the development of GVHD. The majority of these are directed at reducing the incidence of severe acute GVHD, as this is the strongest risk factor for the development of chronic GVHD, which has a significant impact on non-relapse mortality [84, 85].

The most commonly used agents to prevent GVHD are the calcineurin inhibitors cyclosporin and tacrolimus. These drugs are prescribed during the first 3 months following HCST and gradually tapered if there are no further signs of acute or chronic GVHD.

Newer strategies for prevention of GVHD have focused on T-cells, as these are necessary to cause the disease [84]. Depletion of T-cells in the donor graft can lead to significantly lower rates of GVHD but has risks of delayed immune reconstitution in the recipient and associated infective complications [86]. Techniques used for T-cell depletion include anti-thymocyte globulin and alemtuzumab (monoclonal antibody to CD52 receptor) [84].

Treatment of active acute GVHD relies primarily on corticosteroids and calcineurin inhibitors, although a wide range of other treatment options are available and are used as second-line treatment in steroid-refractory cases [69].

First-line treatment for chronic GVHD is with systemic corticosteroids and calcineurin inhibitors. Depending on the site of disease activity, a variety of topical and local therapies are also used. Comprehensive reviews exist on this subject [71, 87].

Management of active oral cGVHD is based on symptom control. It is not usually possible to produce a “normal” mucosal appearance therapeutically, and reticular, plaque-like and sclerotic lesions may be present even when the disease is dormant. Systemic corticosteroids and immunosuppressants are required if the oral mucosa is severely ulcerated, limiting oral intake and quality of life. Systemic treatment will usually be prescribed if another organ is also severely affected, particularly the lungs or liver. Otherwise, options for management of oral mucosal cGVHD include topical corticosteroids, topical immunomodulators such as tacrolimus ointment, topical anaesthetics for symptom control and agents used for the management of dry mouth symptoms. Severe, steroid-dependent or steroid-refractory oral cGVHD may also be treated with extracorporeal photophoresis [88]. Consensus documents have recommended that patients with severe oral cGVHD should be referred to an oral medicine team for specialist management [87].

Oral Cancer Risk

Recipients of HSCT are at a significantly elevated risk of oral SCC. In a large cohort study of HSCT recipients, the observed-to-expected ratio for oral SCC was 7.01 [73].

Further analysis has revealed that the strongest predictor of oropharyngeal SCC risk in HSCT recipients is chronic GVHD. Chronic GVHD has been identified to

lead to a three- to fivefold increased risk of SCC [73, 89]. This risk is present in the first 4 years post transplant and is also maintained greater than 5 years post transplant [73].

Immunosuppression is routinely used to prevent severe GVHD following HSCT. Studies have established that the duration of immunosuppression and exposure to certain immunosuppressive agents, particularly azathioprine, are related to the excess risk of oropharyngeal SCC following HSCT [50, 89–91]. Patients with severe chronic GVHD are usually exposed to a greater duration of immunosuppression than those with a milder course. An important case-control study showed that the risk of oral SCC was increased by eight times after greater than 24 months of immunosuppressive therapy for cGVHD [89].

Although beyond the scope of this chapter, effective management of oral cGVHD would appear to be an important factor in reducing the risk of late second malignancies in the oral cavity. Given the significant contribution that the duration of systemic immunosuppression has to the excess risk, it is desirable that optimal topical treatment is provided for oral cGVHD, in order to reduce the need to systemically immunosuppress these patients. Oral medicine and oral surgery specialists have an important role to play in the correct diagnosis of oral symptoms as being related to cGVHD, provision of optimal topical management of symptoms in order to reduce the need for systemic immunosuppression and screening for malignancy.

In view of the significantly elevated risk of SCC, including oral mucosal SCC, in the post-HSCT patient population, it is essential that effective screening measures are in place. The median time to development of oral SCC following HSCT is 6 years, so long-term screening is essential in order to detect malignancies early when they are more amenable to treatment [89]. Patient education plays an important role—patients should be counselled to seek dental or medical attention if they develop new changes or abnormalities in the oral mucosa. Recommendations from international consensus statements on the management of chronic GVHD have recommended that these patients should be referred to an oral medicine service for ongoing oral mucosal surveillance. Six monthly or more frequent oral mucosal examinations are recommended for patients at high risk of developing SCCs, such as those with severe cGVHD or Fanconi anaemia [81, 87, 92].

HIV

Introduction

Human immunodeficiency virus (HIV) infection is a known risk factor for a number of cancers. Kaposi sarcoma, non-Hodgkin lymphoma and cervical cancer are referred to as “AIDS-defining” cancers, signifying a progression to the acquired immunodeficiency syndrome (AIDS) state. However it has been increasingly recognised that HIV-positive individuals are at increased risk of a number of other

cancers, including anal carcinoma, lung cancer, hepatocellular carcinoma and Hodgkin lymphoma [93]. A number of case reports and case series have also reported an increased risk and demographically different behaviour of oral squamous cell carcinoma in HIV-positive individuals [94–97].

Research into the increased risk of malignancy in HIV has focused on the nature of HIV as an immunodeficiency state, comparable to iatrogenic immunosuppression; on the role of infective agents in the aetiology of malignancy in HIV-positive individuals; and on the contribution of highly active antiretroviral therapy (HAART) to malignancy risk.

Epidemiology

HIV infection is a major worldwide health issue but is particularly problematic in the developing world, where rates of infection continue to rise. UNAIDS data from 2015 estimate that 36.7 million people worldwide were living with HIV, 2.1 million people were newly infected with HIV in 2015, and 1.1 million people died from AIDS in the same year. The highest prevalence of HIV was in Africa [98].

In the United Kingdom in 2015, over 88,000 people were living with HIV and accessing care. There were 6095 new diagnoses of HIV during 2015. The age distribution of people seeking care for HIV is changing, with one in three people accessing care aged 50 years or over, compared to one in seven in 2005 [99].

Pathophysiology

HIV is a retrovirus, from the lentivirus group of this family. Two types have been identified—HIV-1 is the most common, and HIV-2 infection is confined to some areas of Europe and Western Africa. The virus consists of a lipid coat with glycoprotein gp120 expressed on the surface, mediating attachment to host CD4 cells. The virus contains single-stranded RNA, a core protein known as p24, a protease and a reverse transcriptase polymerase. Reverse transcriptase converts viral RNA into DNA, enabling it to be added to the host cell genome.

Following entry of the virus to the host, the gp120 glycoprotein binds to host CD4 T-cells and enters the host cell. Viral reverse transcriptase transforms the viral RNA into double-stranded DNA, which is integrated into the host cell genome. During subsequent transcription, the host cell machinery will produce viral mRNA, which then undergoes translation into the viral structural proteins gp160, p24 and p17. The enzymes protease and reverse transcriptase, along with the structural proteins and the viral RNA, are assembled into the viral capsid which is then budded from the host cell, forming a virion capable of further infection (Ref Kumar and Clark).

During this process, CD4 T-cells are destroyed by direct viral effects, as well as by CD8 cells and NK cells as part of the host immune response. This leads to a gradual depletion of host CD4 T-cells and a resultant immunodeficiency state. Immune activation also occurs which leads to increased numbers of CD4 cells becoming infected and destroyed and an increased susceptibility to inflammation and infection due to damaged mucosal surfaces.

As the disease progresses, the CD4 T-cell count will progressively drop. As this occurs, the prevalence of opportunistic infections increases. At a CD4 T-cell count of less than 200 cells/mm^3, the patient is at a high risk of AIDS-defining illnesses. These are primarily severe and/or unusual opportunistic infections; however the malignancies Kaposi sarcoma and some forms of lymphoma are considered AIDS-defining conditions.

Clinical Features

Primary HIV infection refers to the period between initial infection and the development of antibodies against the virus. During this period, the individual may develop symptoms of HIV seroconversion illness, which includes fever, lymphadenopathy, a maculopapular skin rash and myalgia. This is typically a self-limiting illness and has a wide range of differential diagnoses [100].

Following primary infection many individuals will remain asymptomatic for a period of time. As the viral load rises and the CD4 count decreases, more symptoms and signs will become evident, mainly related to opportunistic infections. If untreated, the median time to development of AIDS is 10 years. Persistent generalised lymphadenopathy may occur during this time.

Prior to the development of AIDS, the infection may become symptomatic. Symptomatic HIV-related conditions include systemic symptoms of fever, fatigue, diarrhoea and weight loss; infections including oropharyngeal candidiasis, herpes zoster and pelvic inflammatory disease; hairy leukoplakia; cervical dysplasia; peripheral neuropathy; and idiopathic thrombocytopenic purpura [100].

The development of AIDS is characterised by a significant decrease in the number of CD4 T-cells and a dysregulation of other elements of the immune system. Clinical manifestations of AIDS are listed in Box 7.1—these conditions are referred to as AIDS-defining conditions, and the presence of these diagnoses in conjunction with the CD4 T-cell count is used to define the progression to AIDS.

The development of highly active antiretroviral therapy (HAART) has changed the outcome of HIV infection dramatically. The condition is no longer considered universally fatal and the progression to AIDS inevitable; rather it is viewed as a chronic disease, which can be effectively managed with medications. Many of the symptomatic features of HIV infection as listed above will reverse with effective HAART. Successful treatment results in a low or undetectable viral load, rising CD4 T-cell count and improvement in symptomatic HIV disease manifestations.

Box 7.1 Centers for Disease Control and Prevention (CDC) AIDS-Defining Conditions [101]

- Bacterial infections, multiple or recurrent (in children aged <6 years)
- Candidiasis of the bronchi, trachea or lungs
- Candidiasis of the oesophagus
- Cervical cancer, invasive
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (>1 month's duration)
- Cytomegalovirus disease (other than the liver, spleen or nodes), onset at age >1 month
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy attributed to HIV
- Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis or oesophagitis (onset at age >1 month)
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (>1 month's duration)
- Kaposi sarcoma
- Lymphoma, Burkitt (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of the brain
- *Mycobacterium avium* complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
- *Mycobacterium tuberculosis* of any site, pulmonary, disseminated or extrapulmonary
- *Mycobacterium*, other species or unidentified species, disseminated or extrapulmonary
- *Pneumocystis jirovecii* pneumonia
- Pneumonia, recurrent
- Progressive multifocal leukoencephalopathy
- *Salmonella* septicaemia, recurrent
- Toxoplasmosis of the brain, onset at age >1 month
- Wasting syndrome attributed to HIV

HAART consists of a combination of medications which target different aspects of the viral lifecycle. The four major classes of drugs, their mechanisms of action, and some examples are listed in Table 7.1. HAART typically comprises at least two different reverse transcriptase inhibitors and at least one drug from another class. Combination therapy is required in order to avoid the generation of resistant strains of the HIV. HIV lacks error-correcting mechanisms for the process of reverse transcription, and this as well as its rapid replication rate leads to a high rate of

Table 7.1 Antiretroviral drugs [100]

Drug class	Mechanism of action	Examples
Entry inhibitors	Inhibit the entry of HIV to CD4 T-cell by preventing fusion and blocking chemokines involved in binding	Enfuvirtide Maraviroc
Nucleoside/nucleotide reverse transcriptase inhibitors	Inhibit transcription of viral RNA into DNA	Zidovudine Lamivudine Abacavir Tenofovir Emtricitabine
Non-nucleoside reverse transcriptase inhibitors	Inhibit transcription of viral RNA into DNA	Efavirenz Etravirine
Integrase inhibitors	Inhibit integration of viral DNA into host cell genome	Raltegravir
Protease inhibitors	Inhibit virion assembly prior to release from the host cell	Atazanavir Darunavir Ritonavir

mutation. This can lead to the generation of resistance very quickly. As such, compliance with HAART is particularly important, and resistance patterns are carefully monitored by HIV specialists.

Oral Cancer Risk

Data regarding the risk of head and neck squamous cell carcinoma in HIV-positive individuals has come mainly from small case series and analysis of registry data in epidemiological studies.

Epidemiological studies based on analysis of registry data have suggested a 2–4× increased risk of oropharyngeal cancer in HIV-positive individuals. These studies have also been unable to demonstrate a significant change in the incidence of oropharyngeal cancer in this patient population following the introduction of HAART [102–105].

Case series have reported that HNSCC tends to occur at a younger age (median 45 years in one study) and at a more advanced stage in HIV-positive individuals [94–96, 106, 107].

The contribution of known HNSCC risk factors such as smoking and alcohol to the development of cancer in HIV-positive individuals has been debated in the literature. Historically, studies have shown an increased rate of tobacco smoking in the HIV-positive cohort; however a meta-analysis failed to demonstrate a difference in the risk of smoking-related cancers between an HIV-positive population and an immunosuppressed population with a lower exposure to tobacco smoking [93]. Studies investigating the increased incidence of lung cancer in the HIV-positive population have established that the excess risk is not entirely attributable to smoking exposure [108].

The introduction of HAART has significantly changed the outlook following HIV diagnosis. HIV-positive individuals on HAART have an increased life expectancy and a lower rate of development of AIDS. This has led to a change in the range of clinical presentations, particularly related to cancer, in people living with HIV [109].

Theories regarding the aetiopathogenesis of HNSCC in HIV-positive individuals have focused on the roles played by immunosuppression, infection and exposure to risk factors.

A large study comparing rates of cancer in an HIV-positive population and an immunosuppressed population (transplant recipients) found a consistent pattern of increased risk of cancer of a variety of sites in both populations [93]. There were very few differences between the two groups in the prevalence of cancer types, apart from Kaposi sarcoma, liver and anal cancers, which were more prevalent in the HIV group. The authors comment that there was a clear increase in the risk of cancers which are definitely or theoretically linked to infection, such as Kaposi sarcoma (human herpes virus 8), lymphoma (Epstein-Barr virus) and cancer of the cervix and anus (human papilloma virus). This increase occurred to a similar extent in both the HIV-positive and post-transplant groups. However there were also increased incidences of cancers not linked to a known infective agent. The authors note that the exposure to risk factors for HNSCC such as smoking was very different in the two study groups, with transplant recipients having a smoking rate similar to the general population but the HIV-positive group having doubled the population smoking rate. Despite this, there was not a significant difference in rates of smoking-related cancers in between the groups. This study concluded that immunosuppression and its effects on immunity to oncogenic viruses play a very significant role in the risk of cancer in HIV-positive individuals.

Another study considering cancer incidence in HIV-positive individuals demonstrated an increased risk of HNSCC associated with increasing severity of immunosuppression and progression to AIDS [103].

Many studies have commented on the possible role played by human papilloma virus in the aetiology of HNSCC in HIV-positive individuals. Given the increased prevalence of other HPV-related carcinomas in this patient population, particularly cervical and anal carcinomas, it has been suggested that the increased risk of HNSCC may also be due to this infection [110]. Studies have shown that HPV infection prevalence is significantly higher in HIV-positive individuals, including high-risk strains such as HPV16 [111].

In the general population, an increasing prevalence of HPV-positive HNSCC has been observed, particularly oropharyngeal squamous cell carcinomas. High-risk viral strains such as HPV16 have been detected in these carcinomas. However the same trend has not been observed in oral cavity carcinomas, which have a lower prevalence of HPV positivity [112].

Despite a higher prevalence of HPV positivity and a modestly increased rate of HNSCC in the HIV-positive population, studies have not been able to demonstrate a definite relationship between HPV positivity and the increased risk of HNSCC in this cohort [110, 113].

A small case series in Japan reported that HIV-positive individuals had an increased risk of developing oral mucosal changes which were considered prema-

lignant and that this risk was the same as that conferred by smoking in their control population [114].

Overall, there is a paucity of information regarding the aetiopathogenesis of HNSCC in HIV-positive individuals. This malignancy is still relatively rare in this patient population, and very large prospective studies are required in order to establish clearer information regarding the condition. In addition, the majority of published data to date is from HIV registries in the developed world. It is not clear whether similar rates of HNSCC occur in the countries with the highest HIV prevalence.

Syphilis

Introduction

Syphilis is a sexually transmitted infection caused by the spirochaetal bacterium *Treponema pallidum*. Acquired infection is transmitted through direct person-to-person sexual contact. Vertical transmission from the mother to the baby causes a congenital infection. Most sexual transmission of syphilis occurs from the genital and mucous membrane lesions of primary and secondary syphilis. Syphilis has often been described as the great imitator because many of the symptoms and signs are difficult to distinguish from other diseases [115].

Epidemiology

Syphilis is a common, worldwide sexually transmitted disease, with approximately 10.6 million new cases reported in 2005 and 2008, according to the World Health Organization. Rates of syphilis infection have been rising over recent years, commonly as a co-infection with other sexually transmitted infections. In the United Kingdom over the period 2003–2012, the number of new diagnoses of syphilis in men rose by 61% and decreased in women by 16%. A recent report regarding the rate of syphilis infections in London in 2015 stated that from 2010 to 2015, the rate of syphilis infection in the city has risen by 163%. The majority of these cases are in men who have sex with men [116].

Pathophysiology and Clinical Features

The first stage of infection with *T. pallidum* is known as primary syphilis and represents local infection at the site of inoculation of the organism. The average incubation time is 2–3 weeks after which a painless papule appears at the site of inoculation.

The primary chancre will heal within 3–6 weeks. Systemic dissemination of *T. pallidum* occurs during the primary stage of infection.

Secondary syphilis develops within 4–6 weeks after the primary infection. This features a variety of systemic signs and symptoms and general malaise.

The third stage of untreated syphilis is termed latent syphilis. This is the period during which patients are asymptomatic but serologically positive.

The fourth stage is referred to as tertiary syphilis. Tertiary syphilis can arise as early as 1 year after initial infection or up to 30 years later. It may involve the central nervous system, cardiovascular system, skin or mucous membranes.

Syphilis is accompanied by additional sexually transmitted diseases in approximately 10% of cases, and syphilis-associated genital ulceration increases the risk of HIV transmission [117].

Clinical Features

Oral syphilitic chancres and mucous patches are usually painless unless they become secondarily infected. The chancre begins as a papule that erodes into a painless ulcer with a smooth, grey surface. A key feature is unilateral lymphadenopathy. Intraoral lesions may present as slightly raised asymptomatic papules with an ulcerated surface and may occur on the lips, tongue and buccal or labial mucosa. Both the chancre and mucous patches resolve spontaneously without treatment; however antibiotic therapy is required to eradicate the systemic infection.

Symptoms of secondary syphilis include generalised rash, fever, lymphadenopathy, malaise, alopecia, aseptic meningitis and uveitis. Maculopapular lesions on the palms and soles occur in 60–80% of patients with secondary syphilis. Up to 58% of patients will have mucocutaneous or mucosal lesions, mucous patches or condylomata lata (broad-based verrucal plaques) in the oral or genital regions [117]. Many other types of oral lesions associated with secondary syphilis have been reported, and they can resemble hairy leukoplakia, erythema multiforme, lichen planus and pemphigus vulgaris (Mignogna et al. 2009).

The classical lesion of tertiary syphilis is the gumma, a rubbery, ulcerated nodule. It is non-infectious and may involve the skin, mucous membranes, skeletal system and viscera. The gumma is a painless lesion that may become secondarily infected. These lesions may occur on the hard palate and can destroy this structure. Atrophic glossitis, the result of contracture of the tongue musculature after healing of a gumma, is viewed as a premalignant lesion [117].

The diagnosis of syphilis is made based on clinical presentation, examination of biopsy tissue using dark-field microscopy or polymerase chain reaction (PCR) and serological tests. Serological testing for syphilis is complex, and the reader is directed to recent guidance documents regarding the most appropriate mode of testing in particular patient groups [118].

Syphilis may be cured with appropriate antibiotic therapy, and these patients should be managed in specialist infectious disease clinics. Prompt diagnosis and antibiotic therapy are important due to the risk of further transmission of the disease and also to prevent long-term complications such as neurosyphilis and involvement of the cardiovascular system [118]. Intramuscular benzathine penicillin G is the first-line drug treatment for all stages of syphilis. The form of penicillin, dose and duration of treatment are determined by the stage and clinical manifestations of the disease. Oral doxycycline may be used in patients with a penicillin allergy.

Treatment may follow positive diagnostic test results or may be empirical. Empirical therapy should be considered in those with suspected early infection (a rash or ulceration) before serology results are available. Sexual contacts of patients with confirmed syphilis should be screened and offered presumptive treatment if follow-up may be problematic. The benefits of empirical therapy (prompt therapy) and risks (potentially unnecessary treatment) should be discussed with the patient.

Oral Cancer Risk

Tertiary syphilis may cause leukoplakic lesions, particularly of the tongue, and this lesion may undergo malignant transformation. This risk appears to persist even when adjusted for alcohol and tobacco exposure [119, 120]. Case series have reported a fourfold increase in SCC related to these lesions. However, these case reports are relatively old, and the risk may be attributable to the agents formerly used to treat the disorder, such as arsenic and heavy metals [117].

In one study of 63 patients with tongue neoplasia, 8% displayed serological positivity, leading the authors to suggest that syphilis serology screening should be routinely performed in patients diagnosed with oral cancer [121]. However it should be noted that in current practice, seropositivity for syphilis is uncommon amongst patients with oral malignancy [122].

Sideropenic Dysphagia (Plummer-Vinson or Patterson-Kelly Syndrome)

Introduction

Sideropenic dysphagia, also known as Plummer-Vinson or Patterson-Brown-Kelly syndrome is characterised by a triad of dysphagia, upper oesophageal web formation and iron deficiency anaemia. It is considered to be a premalignant condition and is associated with cancers of the upper digestive tract.

Epidemiology

This condition most commonly affects females in the fourth to sixth decade of life, although the syndrome has also been described in adolescents and children. Exact epidemiological data is not available; however the syndrome appears to be extremely rare [123].

Pathophysiology

The aetiopathogenesis of Plummer-Vinson syndrome is unknown, but it appears that the most important aetiological factor is iron deficiency. Other possible factors include malnutrition, genetic predisposition or autoimmune processes. Myasthenic changes occur in the muscles involved in swallowing due to the depletion of iron-dependent oxidative enzymes [123]. Atrophy of the oesophageal mucosa and formation of oesophageal webs are common mucosal manifestations.

An autoimmune mechanism has also been proposed, as the syndrome is frequently observed in association with rheumatoid arthritis, thyroiditis, coeliac disease and pernicious anaemia. Other factors such as nutritional deficiency and genetic predisposition are thought to play roles in the causation of the disorder [123].

Clinical Presentation

The dysphagia usually presents intermittently or progressively over years and is painless. Epithelial changes include koilonychia, atrophic glossitis, xerostomia and atrophic changes in the conjunctiva as well as the formation of post-cricoid oesophageal webs. Oral manifestations include stomatitis, angular cheilitis, glossitis and differential atrophy of the fungiform and filiform papilla, recurrent aphthous stomatitis, oral candidosis, erythematous mucositis and a burning mouth. Filiform papillae are the most susceptible to nutritional deficiency and disappear first, followed by the fungiform papillae. Regeneration of the papillae occurs in reverse order, but the vallate and foliate papillae on the posterior third are spared [123].

Diagnosis of iron deficiency anaemia relies on history, clinical examination and appropriate investigations. Radiographic and endoscopic examination of the pharynx may reveal the presence of oesophageal webs.

Iron deficiency anaemia causes epithelial atrophy, changes in epithelial cell kinetics and decreases the repair capacity of the mucosa. This allows carcinogenic and cocarcinogenic agents to act aggressively, predisposing the entire oral mucosa and oesophageal region to malignancy [124].

Management

Correction of the iron deficiency anaemia is the mainstay of treatment. Iron supplementation may be given orally or parenterally if required. Iron supplementation alone can resolve the dysphagia in many patients. Mechanical dilation of webs or strictures by endoscopy may be required. Regular clinical monitoring of the oral mucosa and endoscopic monitoring of the pharyngeal and oesophageal mucosa are necessary in order to identify potentially malignant lesions [123].

Oral Cancer Risk

Sideropenic dysphagia is a major risk factor for the development of squamous cell carcinoma of the upper gastrointestinal tract, with 3–15% of patients with sideropenic dysphagia developing oesophageal or pharyngeal cancer [125].

There are a few case reports of oral cancers in patients with sideropenic dysphagia; however the aetiopathogenesis of this is likely to be multifactorial [123, 126].

New Medications

Emerging risk factors for the development of HNSCC are drugs developed for treatment of advanced melanoma. The BRAF inhibitors vemurafenib and dabrafenib target mutated BRAF protein in melanoma cells and slow the growth of these tumours. They are licenced for the management of advanced or metastatic melanoma.

Immune checkpoint inhibitors such as PD-1 inhibitors pembrolizumab and nivolumab are monoclonal antibodies against cell-surface proteins involved in cancer cell evasion of antitumour T-cell activity. These drugs are used in the management of advanced or metastatic melanoma and metastatic non-small cell lung carcinoma.

Early studies of the BRAF inhibitors demonstrated an increased risk of cutaneous squamous cell carcinoma [127, 128]. However, since the drugs have been more widely used, a number of case reports of oral mucosal changes which have been linked to these drugs, including hyperkeratotic lesions, extensive inflammatory gingival changes and oral squamous cell carcinoma, have emerged [128–131].

Initially it was thought that these drugs would prolong life expectancy by a relatively short period; however a cohort of patients has emerged in whom the drugs have been successful in suppressing tumour activity, and they have survived for several years on therapy [127]. It is likely that more drug-related epithelial effects may become apparent as this cohort of patients grows, and it is important that a comprehensive oral mucosal screening examination forms part of ongoing therapeutic monitoring for these patients.

Rare Associations

A number of rare genetic disorders have been associated with a possibly increased risk of HNSCC. In many of these, the putative link is based on a small number of case reports. It is difficult to comment definitively on the risk of HNSCC in these situations given the rareness of the genetic disorders and the infrequency of reporting of head and neck malignancies.

HNSCC has been reported in association with Bloom syndrome; keratitis-ichthyosis-deafness syndrome; warts, hypogammaglobulinemia, infections and myelokathexis (WHIM) syndrome [132]; familial atypical multiple mole melanoma (FAMMM) syndrome/FAMMM-pancreatic cancer (FAMMM-PC) syndrome [133] and Gorlin-Goltz syndrome. Some authors suggest an increased frequency of HNSCC in ataxia telangiectasia and Li-Fraumeni syndromes; however there is little evidence in the literature to support this.

Basal cell naevus syndrome (OMIM 109400), also known as Gorlin-Goltz syndrome, is an autosomal dominant disorder caused by mutations in the PTCH1, PTCH2 and SUFU genes, which encode proteins involved in tumour suppression. The syndrome is characterised by multiple basal cell carcinomas, odontogenic keratocysts of the jaws, palmoplantar hyperkeratosis, skeletal and facial abnormalities and ectopic intracerebral calcification [134]. Three case reports of squamous cell carcinoma in patients with this disorder exist in the literature [135–137]. Some debate exists as to whether these lesions arise within odontogenic keratocysts, one of the primary manifestations of this syndrome, or form a separate part of the disorder.

Keratitis-ichthyosis-deafness syndrome (OMIM 148210) is due to sporadic or autosomal dominant or recessive inheritance of a mutation in the GJB2 gene, which encodes for a gap junction protein known as connexin 26. Clinically it is characterised by a variety of cutaneous abnormalities, sensorineural deafness, ocular abnormalities, immunodeficiency and mucocutaneous candidiasis and malignancy (squamous cell carcinoma and trichilemmal tumours) [138]. The literature contains four case reports of oral squamous cell carcinoma in patients with this syndrome [139–142]. Cutaneous squamous cell carcinoma is significantly more common [138].

Bloom syndrome (OMIM 210900) is an autosomal recessive inherited disorder with clinical features of short stature, cutaneous abnormalities including photosensitivity and telangiectasia, chromosomal instability, immunodeficiency and cancer predisposition. Due to the chromosomal instability, patients are at risk of accumulating somatic mutations which leads to the increased cancer risk [143]. A number of case reports of HNSCC in this cohort exist [144–146].

Epidermolysis bullosa (EB) is the title given to a heterogeneous group of inherited disorders, generally characterised by skin blistering and erosion. Simplex, junctional and dystrophic forms are recognised, with the dystrophic forms tending towards a more severe phenotype. Dystrophic EB is characterised by severe, widespread blistering of the skin, oesophagus, oral mucosa and conjunctiva, with associated scarring and loss of function. A significant clinical manifestation of EB is the

high rate of cutaneous squamous cell carcinoma, and the prevalence is up to 90% in the severe forms of dystrophic EB [128, 147, 148].

EB is widely reported as being a condition which predisposes to oral squamous cell carcinoma; however it is difficult to support this from the literature, with only a small number of cases reported [128–131, 147, 149, 150].

References

1. Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. N Engl J Med. 2004;350(20):2068–79.
2. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Clin Endocrinol Metab. 2006;91(8):2843–50.
3. Husebye ES, Perheentupa J, Rautemaa R, Kämpe O. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. J Intern Med. 2009;265(5):514–29.
4. Betterle C, Greggio NA. Autoimmune polyglandular syndrome type 1. J Clin Endocrinol Metab. 1998;83(4):1049.
5. Shikama N, Nusspaumer G, Holländer GA. Clearing the AIRE: on the pathophysiological basis of the autoimmune polyendocrinopathy syndrometype-1. Endocrinol Metabol Clin N A. 2009;38(2):273–88.
6. Rautemaa R, Hietanen J, Niissalo S, Pirinen S, Perheentupa J. Oral and oesophageal squamous cell carcinoma – a complication or component of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I). Oral Oncol. 2007;43(6):607–13.
7. Kisand K, Bøe Wolff AS, Podkrajšek KT, Tserel L, Link M, Kisand KV, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med. 2010;207(2):299–308.
8. Puel A, Döffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med. 2010;207(2):291–7.
9. Perheentupa J. APS-I/APECED: the clinical disease and therapy. Endocrinol Metabol Clin N A. 2002;31(2):295–320. vi.
10. Shephard MK, Schifter M, Palme CE. Multiple oral squamous cell carcinomas associated with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(6):e36–42.
11. Bockle BC, Wilhelm M, Muller H, Gotsch C, Sepp NT. Oral mucous squamous cell carcinoma—an anticipated consequence of autoimmune polyendocrinopathy- candidiasis-ectodermal dystrophy (APECED). J Am Dermatol. 2010;62(5):864–8.
12. O'Grady JF, Reade PC. Candida albicans as a promoter of oral mucosal neoplasia. Carcinogenesis. 1992;13(5):783–6.
13. Uittamo J, Siikala E, Kaihovaara P, Salaspuro M, Rautemaa R. Chronic candidosis and oral cancer in APECED-patients: production of carcinogenic acetaldehyde from glucose and ethanol by Candida albicans. Int J Cancer. 2009;124(3):754–6.
14. Rautemaa R, Richardson M, Pfaller M, Koukila-Kahkola P, Perheentupa J, Saxen H. Decreased susceptibility of Candida albicans to azole antifungals: a complication of long-term treatment in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients. J Antimicrob Chemother. 2007;60(4):889–92.
15. Schiødt M. Oral discoid lupus erythematosus. II. Skin lesions and systemic lupus erythematosus in sixty-six patients with 6-year follow-up. Oral Surg Oral Med Oral Pathol. 1984;57(2):177–80.

16. Tebbe B. Clinical course and prognosis of cutaneous lupus erythematosus. Clin Dermatol. 2004;22(2):121–4.
17. Siegel M, Lee SL. The epidemiology of systemic lupus erythematosus. Semin Arthritis Rheum. 1973;3(1):1–54.
18. Santiago-Casas Y, Vila LM, McGwin GJ, Cantor RS, Petri M, Ramsey-Goldman R, et al. Association of discoid lupus erythematosus with clinical manifestations and damage accrual in a multiethnic lupus cohort. Arthritis Care Res. 2012;64(5):704–12.
19. Miot HA, Bartoli Miot LD, Haddad GR. Association between discoid lupus erythematosus and cigarette smoking. Dermatology. 2005;211(2):118–22.
20. Menzies S, O'Shea F, Galvin S, Wynne B. Oral manifestations of lupus. Ir J Med Sci. 2018;187:91.
21. Savage NW, Boras VV, Zaini ZM. Oral squamous cell carcinoma with discoid lupus erythematosus. Oral Oncol Extra. 2006;42(1):32–5.
22. Achtman JC, Werth VP. Pathophysiology of cutaneous lupus erythematosus. Arthritis Res Ther. 2015;17:182.
23. Liu W, Shen Z-Y, Wang L-J, Hu Y-H, Shen X-M, Zhou Z-T, et al. Malignant potential of oral and labial chronic discoid lupus erythematosus: a clinicopathological study of 87 cases. Histopathology. 2011;59(2):292–8.
24. Frohnmayer D, Frohnmayer L, Guinan E, et al., editors. Fanconi anemia: guidelines for diagnosis and management. 4th ed. Eugene, OR: Fanconi Anemia Research Fund, Inc; 2014. http://fanconi.org/index.php/publications/guidelines_for_diagnosis_and_management. Accessed 8 Mar 2017.
25. Mamrak NE, Shimamura A, Howlett NG. Recent discoveries in the molecular pathogenesis of the inherited bone marrow failure syndrome Fanconi anemia. Blood Rev. 2017;31:93.
26. Joenje H, Patel KJ. The emerging genetic and molecular basis of Fanconi anaemia. Nat Rev Genet. 2001;2(6):446–57.
27. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood. 2003;101:1249.
28. Kutler DI, Patel KR, Auerbach AD, Kennedy J, Lach FP, Sanborn E, et al. Natural history and management of Fanconi anemia patients with head and neck cancer: a 10-year follow-up. Laryngoscope. 2015;126(4):870–9.
29. Romick-Rosendale LE, Lui VWY, Grandis JR, Wells SI. The Fanconi anemia pathway: Repairing the link between DNA damage and squamous cell carcinoma. Mutat Res. 2013;743-744:78–88.
30. Smith AR, Wagner JE. Current clinical management of Fanconi anemia. Expert Rev Hematol. 2014;5(5):513–22.
31. Rosenberg PS. Risk of head and neck squamous cell cancer and death in patients with Fanconi anemia who did and did not receive transplants. Blood. 2005;105(1):67–73.
32. Schneider M, Chandler K, Tischkowitz M, Meyer S. Fanconi anaemia: genetics, molecular biology, and cancer - implications for clinical management in children and adults. Clin Genet. 2014;88(1):13–24.
33. Auerbach AD. Fanconi anemia and its diagnosis. Mutat Res. 2009;668(1-2):4–10.
34. Scheckenbach K, Wagenmann M, Freund M, Schipper J, Hanenberg H. Squamous cell carcinomas of the head and neck in fanconi anemia: risk, prevention, therapy, and the need for guidelines. Klin Padiatr. 2012;224(03):132–8.
35. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, et al. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. Arch Otolaryngol Head Neck Surg. 2003;129(1):106–12.
36. Rosenberg PS. Cancer incidence in persons with Fanconi anemia. Blood. 2002;101(3):822–6.
37. Alter BP. Cancer in Fanconi anemia, 1927-2001. Cancer. 2003;97(2):425–40.
38. Reinhard H, Peters I, Gottschling S, Ebell W, Graf N. Squamous cell carcinoma of the tongue in a 13-year-old girl with Fanconi anemia. J Pediatr Hematol Oncol. 2007;29(7):488–91.
39. Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head

and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J Natl Cancer Inst. 2007;99(10):777–89.
40. Iype EM, Pandey M, Mathew A, Thomas G, Sebastian P, Nair MK. Oral cancer among patients under the age of 35 years. J Postgrad Med. 2001;47(3):171–6.
41. Amsterdam JT, Strawitz JG. Squamous cell carcinoma of the oral cavity in young adults. J Surg Oncol. 1982;19(2):65–8.
42. Birkeland AC. Postoperative clinical radiosensitivity in patients with fanconi anemia and head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2011;137(9):930.
43. Alter BP, Joenje H, Oostra AB. Fanconi anemia: adult head and neck cancer and hematopoietic mosaicism. Arch Otolaryngol Head Neck Surg. 2005;131:635.
44. Masserot C, Peffault de Latour R, Rocha V, Leblanc T, Rigolet A, Pascal F, et al. Head and neck squamous cell carcinoma in 13 patients with Fanconi anemia after hematopoietic stem cell transplantation. Cancer. 2008;113(12):3315–22.
45. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol. 2010;11(8):781–9. Available from: http://www.sciencedirect.com/science/article/pii/S1470204510700176.
46. Park JW, Pitot HC, Strati K, Spardy N, Duensing S, Grompe M, et al. Deficiencies in the fanconi anemia DNA damage response pathway increase sensitivity to HPV-associated head and neck cancer. Cancer Res. 2010;70(23):9959–68.
47. Liu GB, Chen J, Wu ZH, Zhao K-N. Association of human papillomavirus with Fanconi anemia promotes carcinogenesis in Fanconi anemia patients. Rev Med Virol. 2015;25(6):345–53.
48. Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst. 2003;95(22):1718–21.
49. van Zeeburg HJT, Snijders PJF, Wu T, Gluckman E, Soulier J, Surralles J, et al. Clinical and molecular characteristics of squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst. 2008;100(22):1649–53.
50. Curtis RE, Rowlings PA, Deeg HJ, Shriner DA, Socie G, Travis LB, et al. Solid cancers after bone marrow transplantation. N Engl J Med. 1997;336(13):897–904.
51. Savage SA, Alter BP. Dyskeratosis congenita. Hematol Oncol Clin North Am. 2009;23(2):215–31.
52. Savage SA. Dyskeratosis congenita. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean L, et al., editors. GeneReviews®. Seattle, WA: University of Washington; 1993.
53. Bessler M, Wilson DB, Mason PJ. Dyskeratosis congenita. FEBS Lett. 2010;584(17):3831–8.
54. Alter BP, Giri N, Savage SA, Rosenberg PS. Cancer in dyskeratosis congenita. Blood. 2009;113(26):6549–57.
55. Kumar K, Shilpasree AS, Chaudhary M. Oral manifestations and molecular basis of oral genodermatoses: a review. J Clin Diagn Res. 2016;10(5):ZE08–12.
56. Vulliamy T, Dokal I. Dyskeratosis congenita. Semin Hematol. 2006;43(3):157–66.
57. Sakiyama T, Kubo A. Hereditary palmoplantar keratoderma clinical and genetic differential diagnosis. J Dermatol. 2016;43(3):264–74.
58. Hennies HC, Hagedorn M, Reis A. Palmoplantar keratoderma in association with carcinoma of the esophagus maps to chromosome 17q distal to the keratin gene cluster. Genomics. 1995;29(2):537–40.
59. Fred HL, Gieser RG, Berry WR, Eiband JM. Keratosis palmaris et plantaris. Arch Intern Med. 1964;113:866–71.
60. Lucker GP, Van de Kerkhof PC, Steijlen PM. The hereditary palmoplantar keratoses: an updated review and classification. Br J Dermatol. 1994;131(1):1–14.
61. Ellis A, Risk JM, Maruthappu T, Kelsell DP. Tylosis with oesophageal cancer: diagnosis, management and molecular mechanisms. Orphanet J Rare Dis. 2015;10:126.
62. Ilhan M, Erbaydar T, Akdeniz N, Arslan S. Palmoplantar keratoderma is associated with esophagus squamous cell cancer in Van region of Turkey: a case control study. BMC Cancer. 2005;5:90.

63. Stevens HP, Kelsell DP, Bryant SP, Bishop DT, Spurr NK, Weissenbach J, et al. Linkage of an American pedigree with palmoplantar keratoderma and malignancy (palmoplantar ectodermal dysplasia type III) to 17q24. Literature survey and proposed updated classification of the keratodermas. Arch Dermatol. 1996;132(6):640–51.
64. Kraemer KH, DiGiovanna JJ. Xeroderma pigmentosum. In: Pagon RA, Adam K, Ardinger HH, Wallace SE, Amemiya A, Bean L, et al., editors. GeneReviews®. Seattle, WA: University of Washington; 1993.
65. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature. 1998;396(6712):643–9.
66. Butt FMA, Moshi JR, Owibingire S, Chindia ML. Xeroderma pigmentosum: a review and case series. J Craniomaxillofac Surg. 2010;38(7):534–7.
67. Fassihi H, Sethi M, Fawcett H, Wing J, Chandler N, Mohammed S, et al. Deep phenotyping of 89 xeroderma pigmentosum patients reveals unexpected heterogeneity dependent on the precise molecular defect. Proc Natl Acad Sci U S A. 2016;113(9):E1236–45.
68. Black JO. Xeroderma pigmentosum. Head Neck Pathol. 2016;10(2):139–44.
69. Dignan FL, Clark A, Amrolia P, Cornish J, Jackson G, Mahendra P, et al. Diagnosis and management of acute graft-versus-host disease. Br J Haematol. 2012;158(1):30–45.
70. Flowers MED, Inamoto Y, Carpenter PA, Lee SJ, Kiem H-P, Petersdorf EW, et al. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood. 2011;117(11):3214–9.
71. Dignan FL, Amrolia P, Clark A, Cornish J, Jackson G, Mahendra P, et al. Diagnosis and management of chronic graft-versus-host disease. Br J Haematol. 2012;158(1):46–61.
72. Zecca M. Chronic graft-versus-host disease in children: incidence, risk factors, and impact on outcome. Blood. 2002;100(4):1192–200.
73. Rizzo JD, Curtis RE, Socie G, Sobocinski KA, Gilbert E. Solid cancers after allogeneic hematopoietic cell transplantation. Blood. 2009;113:1175.
74. Dhir S, Slatter M, Skinner R. Recent advances in the management of graft-versus-host disease. Arch Dis Child. 2014;99(12):1150–7.
75. Mays JW, Fassil H, Edwards DA, Pavletic SZ, Bassim CW. Oral chronic graft-versus-host disease: current pathogenesis, therapy, and research. Oral Dis. 2012;19(4):327–46.
76. Sandmaier BM, Mackinnon S, Childs RW. Reduced intensity conditioning for allogeneic hematopoietic cell transplantation: current perspectives. Biol Blood Marrow Transplant. 2007;13(1 Suppl 1):87–97.
77. Mawardi H, Elad S, Correa ME, Stevenson K, Woo S-B, Almazrooa S, et al. Oral epithelial dysplasia and squamous cell carcinoma following allogeneic hematopoietic stem cell transplantation: clinical presentation and treatment outcomes. Bone Marrow Transplant. 2011;46(6):884–91.
78. Khan FM, Sy S, Louie P, Ugarte-Torres A, Berka N, Sinclair GD, et al. Genomic instability after allogeneic hematopoietic cell transplantation is frequent in oral mucosa, particularly in patients with a history of chronic graft-versus-host disease, and rare in nasal mucosa. Blood. 2010;116(10):1803–6.
79. Janin A, Murata H, Leboeuf C, Cayuela J-M, Gluckman E, Legrès L, et al. Donor-derived oral squamous cell carcinoma after allogeneic bone marrow transplantation. Blood. 2009;113(8):1834–40.
80. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transplant. 2015;21(3):389–401.e1.
81. Treister N, Duncan C, Cutler C, Lehmann L. How we treat oral chronic graft-versus-host disease. Blood. 2012;120(17):3407–18.
82. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-

host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945–56.
83. Filipovich AH. Diagnosis and manifestations of chronic graft-versus-host disease. Best Pract Res Clin Haematol. 2008;21(2):251–7.
84. Choi SW, Reddy P. Current and emerging strategies for the prevention of graft-versus-host disease. Nat Rev Clin Oncol. 2014;11(9):536–47.
85. Lee SJ, Vogelsang G, Flowers MED. Chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2003;9(4):215–33.
86. Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood. 2001;98(12):3192–204.
87. Dignan FL, Scarisbrick JJ, Cornish J, Clark A, Amrolia P, Jackson G, et al. Organ-specific management and supportive care in chronic graft-versus-host disease. Br J Haematol. 2012;158(1):62–78.
88. Greinix HT, van Besien K, Elmaagacli AH, Hillen U, Grigg A, Knobler R, et al. Progressive improvement in cutaneous and extracutaneous chronic graft-versus-host disease after a 24-week course of extracorporeal photopheresis—results of a crossover randomized study. Biol Blood Marrow Transplant. 2011;17(12):1775–82.
89. Curtis RE. Impact of chronic GVHD therapy on the development of squamous-cell cancers after hematopoietic stem-cell transplantation: an international case-control study. Blood. 2005;105(10):3802–11.
90. Leisenring W, Friedman DL, Flowers MED, Schwartz JL, Deeg HJ. Nonmelanoma skin and mucosal cancers after hematopoietic cell transplantation. J Clin Oncol. 2006;24(7):1119–26.
91. Bhatia S, Louie AD, Bhatia R, O'Donnell MR, Fung H, Kashyap A, et al. Solid cancers after bone marrow transplantation. J Clin Oncol. 2001;19(2):464–71.
92. Majhail NS, Rizzo JD, Lee SJ, Aljurf M, Atsuta Y, Bonfim C, et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation. Bone Marrow Transplant. 2012;47(3):337–41.
93. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet. 2007;370:59.
94. Flaitz CM, Nichols CM, Adler-Storthz K, Hicks MJ. Intraoral squamous cell carcinoma in human immunodeficiency virus infection. A clinicopathologic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995;80(1):55–62.
95. Singh B. Alterations in head and neck cancer occurring in HIV-infected patients: results of a pilot, longitudinal, prospective study. Acta Oncol. 1999;38(8):1047–50.
96. Roland JT, Rothstein SG, Mittal KR, Perksy MS. Squamous cell carcinoma in HIV-positive patients under age 45. Laryngoscope. 2006;103(5):509–11.
97. Butt FMA, Chindia ML, Rana F. Oral squamous cell carcinoma in human immunodeficiency virus positive patients: clinicopathological audit. J Laryngol Otol. 2011;126(03):276–8.
98. UNAIDS Core Epidemiology [Internet]. Accessed 29 Mar 2017. Available from: http://www.unaids.org/en/resources/documents/2016/core-epidemiology-slides.
99. England PH. HIV diagnoses, late diagnoses and numbers accessing treatment and care. London: Public Health England; 2016.
100. Adler MW, Edwards SG, Miller RF, Sethi G, Williams I. ABC of HIV and AIDS. London: BMJ Books; 2012.
101. Centers for Disease Control and Prevention. Revised surveillance case definition for HIV infection--United States, 2014. MMWR Recommend Rep. 2014;63(RR-03):1–10.
102. Frisch M, Biggar RJ, Engels EA, Goedert JJ. AIDS-Cancer Match Registry Study Group. Association of cancer with AIDS-related immunosuppression in adults. JAMA. 2001;285(13):1736–45.
103. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer. 2008;123(1):187–94.

104. Clifford GM, Polesel J, Rickenbach M, on behalf of the Swiss HIV Cohort Study, Dal Maso L, Keiser O, et al. Cancer risk in the Swiss HIV cohort study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. J Natl Cancer Inst. 2005;97(6):425–32.
105. Hessol NA, Pipkin S, Schwarcz S, Cress RD, Bacchetti P, Scheer S. The impact of highly active antiretroviral therapy on non-AIDS-defining cancers among adults with AIDS. Am J Epidemiol. 2007;165(10):1143–53.
106. Singh B, Balwally AN, Shaha AR, Rosenfeld RM, Har-El G, Lucente FE. Upper aerodigestive tract squamous cell carcinoma. The human immunodeficiency virus connection. Arch Otolaryngol Head Neck Surg. 1996;122(6):639–43.
107. Butt FMA, Chindia ML, Rana F, Machigo FG. Pattern of head and neck malignant neoplasms in HIV-infected patients in Kenya. Int J Oral Maxillofac Surg. 2008;37(10):907–11.
108. Winstone TA, Man SFP, Hull M, Montaner JS, Sin DD. Epidemic of lung cancer in patients with HIV infection. Chest. 2013;143(2):305–14.
109. Deeken JF, Tjen-A-Looi A, Rudek MA, Okuliar C, Young M, Little RF, et al. The rising challenge of non-AIDS-defining cancers in HIV-infected patients. Clin Infect Dis. 2012;55(9):1228–35.
110. Beachler DC, D'Souza G. Oral human papillomavirus infection and head and neck cancers in HIV-infected individuals. Curr Opin Oncol. 2013;25(5):503–10.
111. Beachler DC, Weber KM, Margolick JB, Strickler HD, Cranston RD, Burk RD, et al. Risk factors for oral HPV infection among a high prevalence population of HIV-positive and at-risk HIV-negative adults. Cancer Epidemiol Biomarkers Prev. 2012;21(1):122–33.
112. Chaturvedi AK. Epidemiology and clinical aspects of HPV in head and neck cancers. Head Neck Pathol. 2012;6(S1):16–24.
113. Gillison ML. Oropharyngeal cancer: a potential consequence of concomitant HPV and HIV infection. Curr Opin Oncol. 2009;21(5):439–44.
114. Chikumaru H, Okubo M, Ueda A. HIV-infected persons are at high risk of developing precancerous lesions of the oral cavity. Open Forum Infect Dis. 2016;3(Suppl 1):2131.
115. Baughn RE, Musher DM. Secondary syphilitic lesions. Clin Microbiol Rev. 2005;18(1):205–16.
116. Public Health England: Syphilis epidemiology in London [Internet]. Accessed 11 Jun 2017. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/547072/london_syphilis_report.pdf.
117. Little JW. Syphilis: an update. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(1):3–9.
118. Kingston M, French P, Higgins S, McQuillan O, Sukthankar A, Stott C, et al. UK national guidelines on the management of syphilis 2015. Int J STD AIDS. 2016;27(6):421–46.
119. Meyer I, Shklar G. The oral manifestations of acquired syphilis. A study of eighty-one cases. Oral Surg Oral Med Oral Pathol. 1967;23(1):45–57.
120. Wynder EL, Bross IJ. Aetiological factors in mouth cancer. An approach to its prevention. Br Med J. 1957;1(5028):1137–43.
121. Dickenson AJ, Currie WJ, Avery BS. Screening for syphilis in patients with carcinoma of the tongue. Br J Oral Maxillofac Surg. 1995;33(5):319–20.
122. Walker DM, Boey G, McDonald LA. The pathology of oral cancer. Pathology. 2003;35(5):376–83.
123. Samad A, Mohan N, Balaji RVS, Augustine D, Patil SG. Oral manifestations of plummer-vinson syndrome: a classic report with literature review. J Int Oral Health. 2015;7(3):68–71.
124. Dinler G, Tander B, Kalayci AG, Rizalar R. Plummer-Vinson syndrome in a 15-year-old boy. Turk J Pediatr. 2009;51(4):384–6.
125. Messmann H. Squamous cell cancer of the oesophagus. Best Pract Res Clin Gastroenterol. 2001;15(2):249–65.
126. Jessner W, Vogelsang H, Puspok A, Ferenci P, Gangl A, Novacek G, et al. Plummer-Vinson syndrome associated with celiac disease and complicated by postcricoid carcinoma and carcinoma of the tongue. Am J Gastroenterol. 2003;98(5):1208–9.

127. Puzanov I, Amaravadi RK, McArthur GA, Flaherty KT, Chapman PB, Sosman JA, et al. Long-term outcome in BRAF(V600E) melanoma patients treated with vemurafenib: patterns of disease progression and clinical management of limited progression. Eur J Cancer. 2015;51(11):1435–43.
128. Larkin J, Del Vecchio M, Ascierto PA, Krajsova I. Vemurafenib in patients with BRAF V600 mutated metastatic melanoma: an open-label, multicentre, safety study. Lancet Oncol. 2014;15:436.
129. Owosho AA, Randazzo J, Rosen EB, Estilo CL, Huryn JM, Chi P, et al. Squamous cell carcinoma associated with chronic graft versus host disease-like/lichen planus-like lesion of the oral cavity in a patient managed for metastatic melanoma with a PD-1 inhibitor pembrolizumab. Oral Oncol. 2016;63:e1–3.
130. Lloyd-Lavery A, Hodgson T, Coupe N, Bond S, Shah K, Espinosa O, et al. Delayed oral toxicity from long-term vemurafenib therapy. Br J Dermatol. 2016;174(5):1159–60.
131. Vigarios E, Lamant L, Delord JP, Fricain JC, Chevreau C, Barrés B, et al. Oral squamous cell carcinoma and hyperkeratotic lesions with BRAF inhibitors. Br J Dermatol. 2015;172(6):1680–2.
132. Cipriani NA, Blair E, Taxy JB. WHIM syndrome and oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(1):105–8.
133. Vinarsky V, Fine RL, Assaad A, Qian Y, Chabot JA, Su GH, et al. Head and neck squamous cell carcinoma in FAMMM syndrome. Head Neck. 2009;31(11):1524–7.
134. Muzio Lo L. Nevoid basal cell carcinoma syndrome (Gorlin syndrome). Orphanet J Rare Dis. 2008;3(1):32.
135. Nicot R, Denglehem C, Qassemyar A, Raoul G, Ferri J. Squamous cell carcinoma of the maxilla in a patient with basal cell nevus syndrome. J Craniofac Surg. 2014;25(2):662–4.
136. Hasegawa K, Amagasa T, Shioda S, Kayano T. Basal cell nevus syndrome with squamous cell carcinoma of the maxilla: report of a case. J Oral Maxillofac Surg. 1989;47(6):629–33.
137. Moos KF, Rennie JS. Squamous cell carcinoma arising in a mandibular keratocyst in a patient with Gorlin's syndrome. Br J Oral Maxillofac Surg. 1987;25(4):280–4.
138. Coggshall K, Farsani T, Ruben B. Keratitis, ichthyosis, and deafness (KID) syndrome: a review of infectious and neoplastic complications. J Am Acad Dermatol. 2013;69:127.
139. Homeida L, Wiley RT, Fatahzadeh M. Oral squamous cell carcinoma in a patient with keratitis-ichthyosis-deafness syndrome: a rare case. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119(4):e226–32.
140. Lancaster LJ, Fournet LF. Carcinoma of the tongue in a child: report of case. J Oral Surg. 1969;27(4):269–70.
141. Baden HP, Alper JC. Ichthyosiform dermatosis, keratitis, and deafness. Arch Dermatol. 1977;113(12):1701–4.
142. Mazereeuw-Hautier J, Bitoun E, Chevrant-Breton J, Man SYK, Bodemer C, Prins C, et al. Keratitis-ichthyosis-deafness syndrome: disease expression and spectrum of connexin 26 (GJB2) mutations in 14 patients. Br J Dermatol. 2007;156(5):1015–9.
143. Cunniff C, Bassetti JA, Ellis NA. Bloom's syndrome: clinical spectrum, molecular pathogenesis, and cancer predisposition. Mol Syndromol. 2017;8(1):4–23.
144. Berkower AS, Biller HF. Head and neck cancer associated with Bloom's syndrome. Laryngoscope. 1988;98(7):746–8.
145. Wong BJ, Hashisaki GT. Treatment of Bloom syndrome patients: guidelines and report of a case. Otolaryngol Head Neck Surg. 1996;114(2):295–8.
146. Ma B, Corry J, Rischin D, Leong T, Peters LJ. Combined modality treatment for locally advanced squamous-cell carcinoma of the oropharynx in a woman with Bloom's syndrome: a case report and review of the literature. Ann Oncol. 2001;12(7):1015–7.
147. Mallipeddi R. Epidermolysis bullosa and cancer. Clin Exp Dermatol. 2002;27(8):616–23.
148. Pfendner EG, Lucky AW. Dystrophic epidermolysis bullosa. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean L, et al., editors. GeneReviews®. Seattle, WA: University of Washington; 1993.

149. Fine J-D, Mellerio JE. Extracutaneous manifestations and complications of inherited epidermolysis bullosa: part II. Other organs. J Am Acad Dermatol. 2009;61(3):387–402. quiz 403–4.
150. Martinez L, Goodman P, Crow WN. Squamous cell carcinoma of the maxillary sinus and palate in epidermolysis bullosa: CT demonstration: J Comput Assist Tomogr 1992;16(2):317–319.

Chapter 8
Oral Submucous Fibrosis

Divya Mehrotra

Introduction

Oral submucous fibrosis (OSMF) is a chronic, insidious, progressive oral mucosal disease affecting the oral cavity, pharynx, and upper digestive tract, causing stiffness of the oral mucosa, restricted mouth opening, and impaired ability to eat, speak, or care for oral hygiene [1].

The WHO initially defined OSMF as an oral precancerous condition associated with a significantly increased risk of cancer [2]. Later at an Oral Cancer and Precancer Workshop by the WHO in the UK, precancer was referred to as "potentially malignant disorders" as it was noted that all disorders described as precancer eventually do not transform into cancer [3].

Terminology

In Sushruta Samhita, OSMF was described as vidari, a swelling within the throat. It presented as burning, prickling pain, haemorrhage, putrid, and necrosed muscle. Schwartz [4] described it in five Indian women in Kenya and termed it "atrophia idiopathies (tropica) mucosae oris". Joshi in 1953 termed it oral submucous fibrosis (OSMF) [2]. Other names suggested for this condition include "diffuse oral submucous fibrosis", "idiopathic scleroderma of the mouth", "idiopathic palatal fibrosis", and "sclerosing stomatitis". Although Pindborg and Sirsat [5] suggested it to be known as "juxta-epithelial fibrosis", oral submucous fibrosis was the most popular term designated to this disease.

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Historical prevalence: Pindborg, a Danish pathologist and WHO consultant, travelled to Southeast Asia to study tropical diseases, wherein, with his Indian colleagues, he planned field studies and examined over 200,000 Indian villagers over 30 years. They together published the epidemiology and clinicopathological aspects of OSMF in over 30 scientific papers [6].

Geographical Distribution and Prevalence

Studies have shown that the number of cases of OSMF has increased rapidly in India, from an estimated 250,000 cases in 1980 to two million in 1993 and five million in 2002. This happened with the vast usage of newly commercially available areca nut preparation: the pan masala [7]. It was extensively used by the younger generation because of its easy access, low price, and marketing strategies [8]. The increasing use of pan masala, with or without tobacco, seems associated with an earlier age of onset of OSMF.

It has been estimated that up to 20% of the world's population consumes betel nut, so probably the incidence of OSMF is higher than what has been reported [7]. Currently, OSMF is considered a public health issue in India, the UK, and South Africa. With an ever-increasing Indian immigrants to the USA, OSMF would soon be seen in the USA too in the near future [9].

OSMF is predominantly encountered among the populations of Southeast Asia or among people migrating from these countries. The prevalence rates of oral submucous fibrosis in Southeast Asia range from 0.04% to 24.4%, 1.0–3.03% in China [10], 0.15–14.4% in Vietnam [11], 0.086–17.6% in Taiwan [12], and 0.2–1.3% in India [13].

Within India also, the prevalence of OSMF varies and has been reported as 1.3% in North India and 0.55% in South India (12). Studies have reported its prevalence in different cities of India: 0.36% in Ernakulam, Kerala, 0.31% in Trivandrum, 0.04% in Andhra Pradesh, and 0.16% in Gujarat. In cities including Lucknow, Bombay, Bangalore, and Trivandrum, a hospital-based survey recorded prevalence of OSMF as 0.51%, 0.50%, 0.18%, and 1.22%, respectively. The prevalence of OSMF has increased not only in India but also in countries like Taiwan, from 8.3 (per 100,000) in 1996 to 16.2 (per 100,000) in 2013, with men having a significantly higher prevalence than women [14].

Premalignant Potential

Paymaster [15] first described its premalignant potential [2] which has now been observed as 7–30% [16]. The 10-year oral cancer transformation rate for OSMF is much higher than that of leukoplakia, 0%, and OSMF, 11%, and when both are seen together, it is greater than 15%. Combination of leukoplakia and OSMF has a much higher malignant transformation rate [17].

Aetiology

The aetiology of OSMF is thought to be multifactorial, although areca nut is the major risk factor. Areca nut chewing, local irritants (chillies), nutritional deficiency, and autoimmune disease are all held responsible for OSMF. Areca nut is consumed alone or as an ingredient of betel quid. The other ingredients of quid like betel leaf, slaked lime, or tobacco have not been established as causative factor for OSMF.

Association with Areca Nut

The association of areca nut consumption with OSMF is based on studies undertaken in India, Pakistan, Sri Lanka, and Taiwan, which estimate a relative risk (RR) of 1.8–172.

Pakistan

RR was estimated by comparing 157 OSMF patients and 157 controls in Karachi, Pakistan, and was observed as 154 (95% CI 34–693) in areca nut users.

Sri Lanka

A study of 74 OSMF and 74 controls in a hospital-based study confirmed a strong association of OSMF with areca nut used in betel quid (odds ratio: 171.83; 95% confidence interval (CI): 36.35–812.25).

Taiwan

Three forms of betel quid with areca nut were used in Taiwan: betel quid, lao-hwa quid, and stem quid. All reported studies have indicated a significant association between OSMF and areca nut.

China

In a study in China [18], 43% of OSMF patients chewed areca nut alone.

India

Areca nut chewing habit in OSMF patients in India ranges from 34% to 100%; but these habits differ in men and women (men prefer to chew gutkha, mawa, and kharra, while women chew solely areca nut). In Maharashtra, 4.2% of females who chewed areca nut only suffered from OSMF. Men are seen to develop OSMF comparatively at a younger age.

The odds ratio of OSMF at 95% confidence interval ranges from 4.77 to 6.88 with usage of tobacco-less pan masala and 4.55–9.71 with tobacco pan masala. When areca nut was used as mawa (tobacco and lime added), RR was observed as 29.9 [19]. In a study conducted in Lucknow, the OR assessed using multivariate analysis was 14.09 for tobacco-less pan masala and 5.39 for tobacco pan masala [20].

Areca Nut

Arecoline, an areca nut alkaloid, is the main culprit that decreases collagen breakdown and subsequently leads to increased fibrosis causing OSMF. Other active components of areca nut include alkaloids, polyphenols, and trace elements (sodium, magnesium, chlorine calcium, vanadium, manganese, copper, and bromine). Polyphenols of areca nut cause collagen fibres to cross-link, making them less susceptible to collagenase degradation.

Aetiopathogenesis

The pathogenesis of OSMF is believed to be multifactorial: (1) Long-term exposure of areca nut causes fibroblasts to produce a high amount of collagen; (2) decreased secretion of collagenase, due to stabilization of collagen by catechin and tannins; (3) production of stable collagen by fibroblasts; (4) increased collagen cross-linking by upregulation of lysyl oxidase; (5) deficiency in collagen phagocytosis, and (6) deficiencies in micronutrients and vitamins.

- **Changes in extracellular matrix (ECM)**: Areca alkaloids induce contraction of the buccal mucosal fibroblast. Arecoline stimulates connective tissue growth factor production through reactive oxygen species (ROS). It increases the production of tissue inhibitor of metalloproteinase-1 (TIMP). At concentrations of 0.4–0.8 mM, arecoline induces cytotoxicity and apoptosis. Prolonged exposure to arecoline suppresses endothelial cell proliferation, leading to impairment of vascular function, decreased vascularity, and eventually atrophy of the epithelium. Hypoxic environment predisposes the tissue to carcinogenesis.

- **Stage-specific alterations in ECM**: Overexpression of collagen type III in the lamina propria and submucosa is seen in the early-stage OSMF. In the intermediate stage, extensive and irregular deposits of elastin are found around muscle fibres. In the advanced stage, collagen type I dominates the ECM.
- **Matrix metalloproteinases (MMPs) and TIMPs**: A balance between MMPs and TIMPs is mandatory to maintain the normal integrity of connective tissue. In OSMF this equilibrium is disturbed, causing increased deposition of ECM.
- **Inflammatory cytokines and growth factors**: Upregulation of cytokines (transforming growth factor, TGF) triggers increased collagen production and decreased matrix degradation. Downregulation of bone morphogenetic protein 7, a known negative modulator of fibrosis, allows more fibrosis.
- **Epithelial mesenchymal transition (EMT)**: Chemical cell injury produces ROS that triggers both MAPK and NF-kB pathways involved in the EMT, leading to secretion of a variety of inflammatory mediators, such as prostaglandin E2, interleukin-6, TNF-a, and TGF-b. HIF-1a enhances EMT and promotes fibrinogenesis by increasing the expression of lysyl oxidase genes.
- **ROS and apoptosis**: At higher arecoline concentrations, oxidative stress may induce epithelial cell death and apoptosis, while sublethal concentrations upregulate the stress responsive genes.
- **Genetic polymorphisms**: Various chromosomal, genetic, and molecular alterations are associated with the pathogenesis of OSMF. A microarray study in OSMF has shown upregulated 716 genes and downregulated 149 genes. Polymorphisms of various genes like cytochrome P450 and genetic polymorphism of lysyl oxidase may also contribute to an increased susceptibility to OSMF.

Other Factors

Indian habit of chewing "pan" has led to the assumption that it causes OSMF, but it has been seen that many afflicted with OSMF have never even used "pan".

Although the data emerged over the past 15 years has expanded our understating of the aetiological role of areca nut, only 1–2% of the population chewing areca nut develop OSMF. This suggests either a genetic predisposition [17] or sensitization of the oral mucosa by iron and/or vitamin B complex deficiencies. Such conditions are much more commonly seen among Indian females, which explains the higher incidence among females. It is for this reason vitamin and iron deficiencies have been given aetiologic importance in OSMF.

It is suspected that continuous prolonged action of mild irritants, such as capsaicin found in green chillies, is also responsible for OSMF. Alcohol consumption and trauma by sharp teeth may enhance the possibility of OSMF. However, it must be remembered that OSMF has also been reported in patients with no habit of tobacco chewing or smoking.

Autoimmunity

Evidence on OSMF supports an autoimmune aetiology. The high frequency of HLA haplotypic pairs in OSMF and scleroderma suggests its immunological derangement. There is increasing evidence that immune response genes are related to HLA complex.

Role of Copper in Pathogenesis of OSMF

- There is a high copper content in areca nut as well as in saliva and serum of areca nut chewers, which suggests its role in OSMF. These copper levels vary in mild OSMF to severe. The enzyme lysyl oxidase, copper-dependent and critical for collagen cross-linking and organization of ECM, appears to be responsible for causing OSMF in areca nut chewers.
- A raised concentration of copper in drinking water stimulates the activity of lysyl oxidase leading to fibrinogenesis [21]. Although copper is rarely found in drinking water, home corrosion of copper piping can contaminate drinking water. This suggests development of OSMF in low socioeconomic strata in the developing countries.

Salivary Pooling

Sites of areca nut chewing and saliva collecting play an important role in the occurrence of OSMF. As the lower lip is a favourable site to place pan masala, and also saliva pools here due to gravity, fibrosis occurs in the lower lip [22].

Symptoms

Prodromal Symptoms

The onset of the condition is insidious and presents as a burning sensation in the mouth on consumption of spicy food. Other early symptoms are blisters, ulcerations, or recurrent stomatitis. Vesicles may be found in the soft palate, anterior faucial pillars, buccal mucosa, or lower lip. Increased salivation, abnormal gustatory sensation, and sometimes even dry mouth can present clinically in the early stages of OSMF.

Later Symptoms

Few years later, there is stiffening of the buccal mucosa, leading to restricted mouth opening, difficulty in swallowing, and inability to whistle or to blow candle. Extension of fibrosis into the oropharynx may cause referred pain in ears or deafness due to occlusion of the Eustachian tubes. Vesicles may sometimes appear even in the later phase. Sometimes a nasal voice may also be observed due to involvement of the nasopharynx.

Clinical Features

- Males are affected more common than females, though both sexes are at risk equally.
- Second or third decade usually but even reported in as early as 8-year-old boy.
- Facial appearance shows sunken cheeks (Fig. 8.1).
- Vesicles develop in the soft palate, anterior faucial pillars, buccal mucosa, or lower lip, with ulcers on rupture. Culture of the vesicular fluid does not reveal any specific organism.
- Oral pain and burning sensation on eating spicy foodstuff.
- Subsequently oral mucosa becomes blanched, opaque, and white, with development of fibrous bands.
- The fibrous bands in buccal mucosa run vertically, while they are circular in the lip, running parallel with the fibres of the orbicularis oris muscle, causing thinning and stiffening of lips. Intense fibres present in the soft palate radiate in an arched manner, from pterygomandibular raphe or anterior faucial pillar across the soft palate to the retromolar area and the base of the tongue.
- Palate and faucial pillars were believed to be the areas initially affected, but it has been observed that the buccal mucosa and lower lip are affected earlier, may be due to the shift of habit from smoking to smokeless tobacco.
- The palate and/or buccal mucosa are the sites with maximum involvement and gingivae and upper lip the least.
- Cheeks may present mottled appearance due to alternating areas of fibrosis and pigmented mucous membrane.
- Vertical fibrous bands may be felt in both cheeks under the mucous membrane, and these can become quite tense when the patient attempts to open mouth.
- Gradual inability to open mouth restricted mouth movements (e.g., eating, whistling, blowing, sucking).
- The soft palate sometimes becomes inelastic and pearly white, with restriction of movements.
- The faucial pillars appear thick, short, and firm, and the palatine tonsils appear pressed between them.

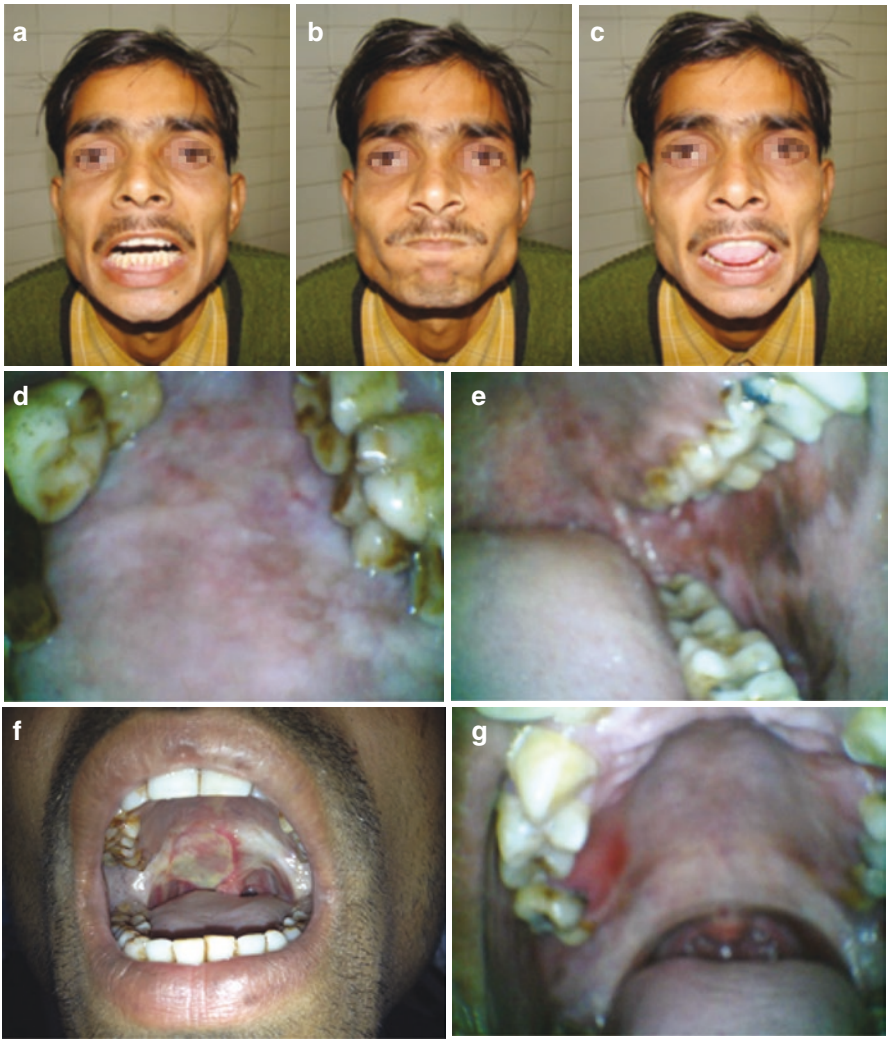


Fig 8.1 Clinical presentation. (**a**) Inability to open mouth. (**b**) Inability to puff cheeks. (**c**) Inability to protrude the tongue. (**d**) Blanched palate. (**e**) Mottled buccal mucosa. (**f**) Blanched soft palate with ulceration. (**g**) Shrunken uvula

- Uvula may be hooked up like a hockey stick, due to fibrosis, appearing budlike and shrunken.
- Impaired tongue movement and sometimes atrophy of the tongue papillae.
- Gingivae and the floor of the mouth may be affected too.
- Oropharynx may appear blanched and indurated.
- Dysphagia to solids (if oesophagus is also involved).
- Xerostomia.
- Altered gustatory sensation.
- Deafness or impaired hearing due to stenosis of the Eustachian tubes.

- Nasal tone.
- Higher incidence of caries and periodontitis due to restriction of access for oral hygiene and dental care.

Laboratory Findings

- ESR raised.
- Anaemia is often present.
- Eosinophilia.
- An increased gamma globulin may be present.

Histological Features [23]

There is epithelial atrophy with juxta-epithelial inflammation. Excessive collagen fibres are seen in lamina propria, submucosa, muscle fibres, and salivary glands.

These histopathologic findings are explained as mucosal changes and submucosal changes:

Mucosal Changes

- Epithelial atrophy with epithelial atypia.
- Loss or sawtoothing of rete ridges and liquefaction degeneration of the basal layer.
- Pigment-containing cells increase in epithelium, and golden-brown pigment granules get scattered in the basal cells and lamina propria.
- Superficial ulceration: The ulceration is replaced by granulation tissue. Signs of secondary infection like necrosis and suppuration can be noted.
- Hyperplastic changes include hyperkeratosis, acanthosis, parakeratosis, basal cell hyperplasia, papillomatosis, and pseudo-epitheliomatous hyperplasia.
- Dysplastic changes include slight variation in size and shape, enlargement of nuclei, prominent nucleoli, and mitotic activity.
- Lamina propria shows fibrosis and hyalinization with a chronic inflammatory infiltrate.

Submucosal Changes

– These are labelled as **mild** if there is early fibrosis, **moderate** if diffuse fibrosis, and **severe** if diffuse fibrosis is with hyalinization and atrophic changes in minor salivary glands and skeletal muscle.
– Increased dilated and congested capillaries.

– Band-like chronic inflammatory infiltrate (lymphocytes, plasma cells, macrophages, neutrophils, and eosinophils) in the upper submucosa.
– Oedema and congestion.
– Subepithelial vesicle formation in some cases.

Radiographic Assessment

An OPG (orthopantomogram) is advised to check for mandibular coronoid process enlargement, as prolonged trismus leads to elongation of the coronoid process.

Classifications

Pindborg [24**]** divided OSMF into three stages:

- **Stage 1**: Stomatitis, erythematous mucosa, vesicles, mucosal ulcers, melanotic mucosal pigmentation, and mucosal petechiae.
- **Stage 2**: Fibrosis occurs in healing vesicles and ulcers.
- **Stage 3**: Leukoplakia in more than 25% of OSMF cases. Speech and hearing deficit due to involvement of the tongue and Eustachian tube.

Mehrotra [25**], clinical grading:**

- **Grade 1**: Stomatitis, burning sensation in buccal mucosa but no detection of fibres
- **Grade 2**: Palpable fibrous bands, involvement of the soft palate, maximum mouth opening 26–35 mm.
- **Grade 3**: Blanched oral mucosa, involvement of the tongue, maximal mouth opening 6–25 mm.
- **Grade 4**: Fibrosis of lips, maximal mouth opening 0–5 mm

Khanna and Andrade [26**]**: Classification based on clinical and histopathological features:

- **Group I**: Very early cases—burning sensation in the mouth, acute ulceration and recurrent stomatitis, and no restriction in mouth opening.
- **Histology**: Fine fibrillar collagen network interspersed with marked oedema, blood vessels dilated and congested, plump young fibroblasts with abundant cytoplasm, and inflammatory cells (polymorphonuclear leukocytes with few eosinophils) aggregated. The epithelium is normal.
- **Group II**: Early cases—buccal mucosa is mottled and marble like, with widespread sheets of palpable fibrous bands, mouth opening of 26–35 mm.
- **Histology**: Juxta-epithelial hyalinization, collagen as thick separate bundles, blood vessels dilated and congested, moderate amount of young fibroblasts,

inflammatory cells (polymorphonuclear leukocytes, few eosinophils, and occasional plasma cells), and flat or short epithelial rete pegs with varied keratinization.

- **Group III**: Moderately advanced cases—trismus, mouth opening 15–25 mm, buccal mucosa pale firm, atrophy of vermilion border, vertical fibrous bands palpable at soft palate, pterygomandibular raphe, and anterior faucial pillars.
- **Histology**: Juxta-epithelial hyalinization, thick collagen bundles, residual edema, constricted blood vessels, mature fibroblasts with scanty cytoplasm and spindle-shaped nuclei, inflammatory exudates (lymphocytes and plasma cells), atrophic epithelium with loss of rete pegs, and muscle fibres with thick and dense collagen fibres.
- **Group IVA**: Advanced cases—severe trismus, mouth opening less than 15 mm, thick faucial pillars, shrunken uvula, restricted tongue movement, and circular band around lip and mouth.
- **Group IVB**: Advanced cases—hyperkeratotic leukoplakia and/or squamous cell carcinoma.
- **Histology**: Collagen hyalinized smooth sheet, extensive fibrosis, obliterated mucosal blood vessels, eliminated melanocytes, absent fibroblasts within the hyalinized zones, total loss of epithelial rete pegs, presence of mild-to-moderate atypia, and extensive degeneration of muscle fibres.

Diagnosis: OSMF is diagnosed on the basis

- Clinical features
- Histological features
- Radiographic features

Management

The management goal is to treat the signs and symptoms, inhibit progression, and reduce risk of malignant transformation, although various treatment methods have been proposed. The current protocol consists of:

- Preventive: discontinuation of habit and counselling
- Medical treatment
- Nutritional supplementation
- Surgical management
- Physiotherapy

Abstinence from the habit of chewing areca nut is the first step towards treatment of OSMF. Instructions should be given to minimize consumption of spicy foods and maintain proper oral hygiene. Explanation about the probable malignant potential and counselling for de-addiction is important. Any sharp teeth or cusps are rounded off to prevent trauma to the cheek. Extraction of all third molars is recommended to avoid undue trauma on the inflamed and atrophied mucosa.

Medical Management

Medical treatment is designed to increase maximal inter-incisal mouth opening and alleviate burning sensation, vesiculation, and fibrosis. Based on the possible aetiopathogenesis, drugs, such as steroids, enzymes, antioxidants, vitamins, and microelements have been advocated to treat OSMF [27].

Steroids

Steroids have been used extensively over the past decades in the treatment of OSMF because of their anti-inflammatory properties. These include short-acting (hydrocortisone), intermediate-acting (triamcinolone), and long-acting (betamethasone and dexamethasone).

Cytokines and growth factors produced by inflammatory cells induce proliferation of fibroblasts, upregulate collagen synthesis, and downregulate collagenase production. Glucocorticoids inhibit the generation of these inflammatory factors and also increase their apoptosis, thereby relieving the symptoms but fail to reverse the abnormal deposition of fibrotic tissues or recover the suppleness of mucosa. Hence, the use of steroids is associated with a high relapse rate and unwanted side effects on prolonged use. Steroids are therefore used as an adjunct therapy [28].

Modalities of Corticosteroids Used in the Treatment [29]

Topical

Triamcinolone acetonide—0.1%
Betamethasone—0.5%

Systemic

Prednisolone—20 mg/day
Dexamethasone—4 mg/day
Triamcinolone—12 mg/day

Intra-lesional

Dexamethasone—4 mg/ml
Triamcinolone—40 mg/ml
Hydrocortisone—25 mg/ml

Short-acting drugs: Hydrocortisone intra-lesional injection 1.5 cm^3 given once a week for a duration of 12 weeks has proven to be beneficial. Systemic corticosteroids are useful in only early and mild cases.

Intermediate-acting drugs: Topical triamcinolone acetonide 0.1% and local injection of triamcinolone acetonide can be used in the early stages of OSMF.

Long-acting drugs: Dexamethasone 4 mg intra-lesional injections are given twice a month for 2–3 months and also if given in combination with hyaluronidase give better long-term results. Betamethasone is given as 4 mg/ml intra-lesional injections biweekly. It is more effective in combination with lycopene or hyaluronidase and vitamin E.

Enzymes

Collagenase is a lysosomal enzyme that can degrade esters, proteins, polysaccharides, and glycosides. Collagenase treatment has been reported to be approximately fivefold more effective than triamcinolone [29]. A 2 mg of collagenase dissolved in 1 ml of distilled water is used for injection and has shown significant improvement in mouth opening and hypersensitivity to spices, sour, cold, and heat. Adverse reactions like pain swelling and trismus may be seen after injections of collagenase which is considered to be an allergic reaction of this agent.

Hyaluronidase has shown a much faster but short-term response in improving the burning sensation and ulceration than dexamethasone. It acts by depolymerizing hyaluronic acid, the ground substance in connective tissue, lowering the viscosity of the intercellular cement, and thus reducing collagen formation. Intra-lesional injections of 1500 IU of hyaluronidase mixed with 2% lignocaine twice daily for 10 weeks have been tried with satisfactory results in improving mouth opening.

Chymotrypsin, an endopeptidase that hydrolyzes ester and peptide bonds, has also been used as a proteolytic and anti-inflammatory agent in the treatment of OSMF. Local injection of chymotrypsin, such as Chymotrypsin (5000 IU), twice weekly as submucosal injections for 10 weeks has proved to be successful in treating OSMF.

Peripheral Vasodilators

Pentoxifylline, a methylxanthine derivative with vasodilating properties and ability to reduce blood viscosity, can suppress leukocyte function, alter fibroblast physiology, and stimulate fibrinolysis. Pentoxifylline 400 mg three times daily, as coated, sustained release tablets, has been used as an adjunct therapy for improvement in mucosal suppleness.

Buflomedil, another vasoactive agent, has shown quicker symptomatic relief but with little improvement in mouth opening.

Nylidrin hydrochloride, a peripheral vasodilator, diffuses fibrosis by relieving the local ischemic effect and helping the nutritional substances to be transported to the affected tissues.

Tea pigments, oxidized polyphenols derived from tea leaves, also decrease blood viscosity and improve microcirculation. Tea-pigment tablets have shown an overall success rate of 58.8% in the treatment of OSMF.

Placental Extracts (Placentrex)

Placentrex, an aqueous extract of human placenta, can be subdivided into four fractions such as aqueous, lipoidal, immune gamma globulins, and tissue coagulants. The aqueous extract of placenta acts as a biogenic stimulator in the cellular metabolism and anti-inflammatory and analgesic, increases blood circulation and tissue vascularity, arrests growth stagnation, and lowers the immune response factor.

Aqueous extract of fresh human placenta contains enzymes, nucleotides, vitamins, amino acids, steroids, fatty acids, and trace elements: cadmium, potassium, calcium, magnesium, copper, iron, phosphorous, and silicone. A 2 cm^3 of placentrax injection intra-lesional at weekly intervals for 10 weeks has been found to be superior to cortisone. A combination of dexamethasone, hyaluronidase, and placental extract gives better results than a single drug.

Antioxidants

Antioxidants reduce the free radical reaction that can cause DNA mutations and changes in lipid peroxidation of cellular membranes and changes in enzymatic activities.

Lycopene, an unsaturated carotenoid that gives red colour to the tomatoes, is a powerful antioxidant, with about twice the potency as of β-carotene, and has shown anti-proliferative properties both in animals and in vitro studies. It has shown to modulate dysplastic changes by inhibition of abnormal fibroblasts, upregulation of lymphocyte resistance to stress, and suppression of inflammatory response. A 16 mg of lycopene daily in 2 divided doses for 2 months or in combination with intralesional injections of betamethasone has shown marked improvement in mouth opening and associated symptoms.

Beta-carotene is the precursor of vitamin A and a powerful antioxidant. Topical application improves the integrity of oral epithelium as well as induces reversal of dysplastic epithelium. Regular intake of beta-carotene combined with routine measures considerably reduces the risk of malignant transformation. Six weeks of beta-carotene and vitamin E tablets taken thrice daily have shown a marked increase in mouth opening and tongue protrusion in OSMF.

Alpha-lipoic acid is a sulphur-containing substance, acts as a coenzyme in the Kreb's cycle, and is claimed to be the near-perfect antioxidant. It has a good potential action of scavenging free radicals and can dissolve in both water and fat. Alpha-lipoic acid 100 mg, 1 capsule per day for 30 days, has shown reduction in burning sensation and improved mouth opening.

Antoxid tablet (containing beta-carotene 50 mg, vitamin A 2500 IU, vitamin E 10 IU, vitamin C, zinc, manganese, and copper) given thrice daily for 6 weeks has been a significant clinical improvement in patients with OSMF.

Recombinant Human Interferon Gamma (γ-IFN)

Interferon-γ (IFN-γ), an anti-fibrosis factor, causes downregulation of fibroblast proliferation and collagen synthesis and upregulation of anti-fibrotic cytokine and collagen synthesis in the basal layer of epithelium and lamina propria. Patients treated with γ-IFN showed improvements in mouth opening of 8 ± 4 mm (42%) [30]. Injections of IFN gamma produce headache, flu-like symptoms, and myalgia. Intra-lesional injection of γ-IFN (0.01–10.0 U/ml) is advocated three times daily for 6 months.

Immunized Milk

Immunized milk is skimmed cow milk, immunized with multiple human intestinal bacteria to gain good anti-inflammatory effect. It contains vitamins A, C, B1, B2, B6, and B12, nicotinic acid, pantothenic acid, folic acid, micronutrients (iron, copper, zinc), and 20–30% higher concentration of IgG type I antibody than in commercial milk. Its anti-inflammatory properties suppress the inflammation and modulate cytokine production. Tai et al. [31] advocated 45 g of immunized cow milk twice daily, for 3 months, and reported 69.2% success by improving the maximum mouth opening by more than 3 mm.

Stem Cell Therapy

Intra-lesional injection of autologous bone marrow stem cells induces local angiogenesis, decreases fibrosis, and improves mouth opening [32, 33].

Herbal Extracts

Turmeric

Turmeric (haldi), a rhizome of *Curcuma longa*, is a yellow-orange spice full of flavour. An orange pulp contained in the rhizome constitutes the source of turmeric medicinal powder. Components of turmeric are named curcuminoids and include curcumin [34]. Curcumin inhibits the products of inflammation such as prostaglandin and leukotrienes by inhibiting cyclooxygenase and lipoxygenase pathways of inflammation. It has a scavenging effect on superoxide free radicals, hydroxyl radicals, and lipid peroxidation; and its fibrinolytic action leads to inhibition of lipid peroxidation, checking cellular proliferation, and inhibition of collagen synthesis.

Hastak [35] studied the effect of turmeric oil (600 mg), its alcohol extracts (3 g), and oleoresin (600 mg) on cytogenetic damage in OSMF after daily intake for 3 months and concluded that turmeric oil with oleoresin acts synergistically in vitro and protects DNA damage.

Aloe vera

Aloe vera is a mannoprotein that contains many wound healing hormones. The polysaccharides in the gel of the leaves have wound healing, anti-inflammatory, anticancer, immuno-modulatory, and gastroprotective properties. It has shown to reduce the burning sensation and gradual recovery of mouth opening. It is relatively safe and can be applied topically in the treatment for OSMF. Topical application of 5 mg of aloe vera thrice daily for 3 months has been reported to reduce burning sensation and improve mouth opening.

Oxitard

Oxitard contains various plant extracts and oils. A dose of 2 oxitard capsules twice daily for a period of 3 months gives significant increase in mouth opening along with decrease in pain [34].

Spirulina

Spirulina, blue-green algae, with a rich natural source of proteins, carotenoids, and other micronutrients [36], has antioxidant properties with high amount of beta-carotene and superoxide dismutase for effective use in OSMF.

Nutritional Support

The rationale of giving nutrients in OSMF patients is to overcome the deficiencies and promote normal cellular processes to protect against carcinogenesis.

1. Supplementing diet with foods rich in vitamins A, B complex, and C and iron.
2. Advice green leafy vegetables, red tomatoes, and fresh fruits.
3. Advice green tea.
4. High-protein diet.
5. Vitamin A is important for maintaining the normal growth and repair of epithelial tissues. It helps in the epithelial differentiation by mucous secretary and keratinization tissues, and in adequate concentration, it delays or even reverses the progress of premalignant cells to cells with invasive malignant potential.
6. Vitamin B complex boosts metabolism, enhances the immune system, and encourages cell growth and division.
7. Vitamin C acts in wound healing, for the integrity of cellular immune responses and anti-inflammatory activities.
8. Minerals like *zinc* are essential for DNA synthesis and cell division and as a constituent of many enzymes. The amount of zinc greatly increases during tissue repair. Moreover, zinc is the antagonist of copper that is released from betel quid to induce lysyl oxidase activity, upregulate collagen synthesis, facilitate collagen

cross-linking, and inhibit collagen degradation. **Magnesium** plays essential roles in stabilizing effects on excitable membranes.

Surgical Management

Surgical treatment is designed to improve the extent of mouth opening and includes excision of fibrous bands, coronoidectomy, and reconstruction of the surgical defect with local or distant flaps. However, recurrence may occur because of secondary contracture of the grafted tissue, resulting in restricted mouth opening.

Surgical measures, such as forceful mouth opening or incising the fibrotic bands under general anaesthesia without any grafting, cause even more fibrosis. Submucosal resection of fibrotic bands and replacement with a graft are the better options, and coronoidectomy, followed by physiotherapy, further improves mouth opening.

Resection of bands: An intraoral incision is made in the buccal mucosa at the level of occlusal plane, 1 cm away from the corner of the mouth to anterior faucial pillars to cut the fibrous bands in the cheek mucosa (Fig. 8.2). After fibrous bands are released, all third molars present are extracted. Maximum mouth opening with the help of Ferguson or mouth gag is recorded.

LASER can also be used for fibrotomy instead of a surgical knife with an advantage of minimal bleeding and no psychological fear of surgery to the patient.

Coronoidectomy: Coronoidectomy with temporal myotomy is recommended to improve mouth opening. The coronoid processes are approached through the same

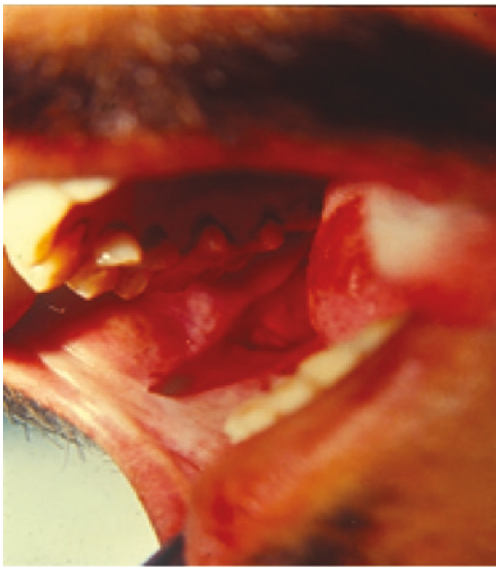


Fig 8.2 Fibrotomy

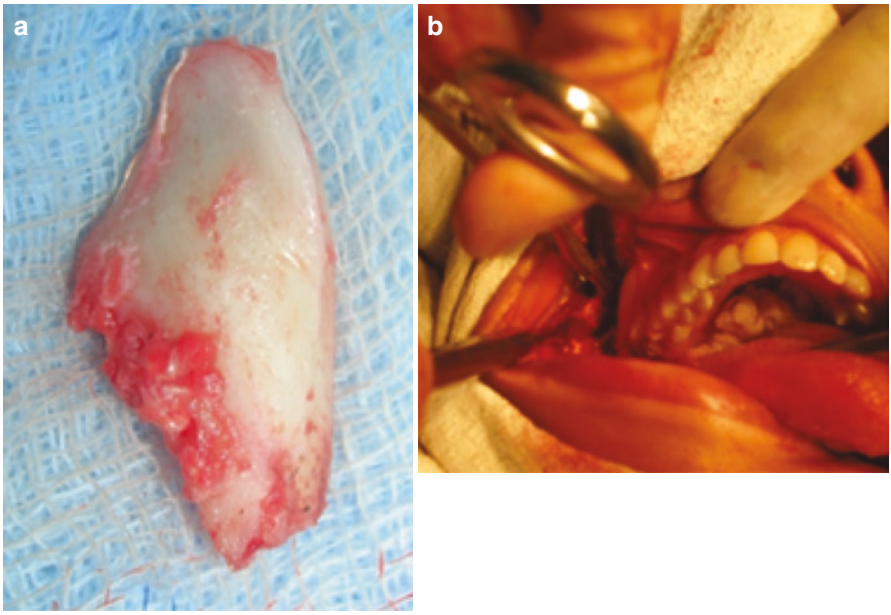


Fig 8.3 (**a**) Coronoidectomy. (**b**) Coronoid process

incision by stripping the temporalis tendon attachment on the anterior border of ramus (Fig. 8.3). Coronoid process is held with forceps, and osteotomy is made extending from the anterior border of ramus to the depth of the sigmoid notch. After completion of osteotomy, coronoid is placed on traction with Kocher's forceps and temporalis muscle, and tendon is detached to facilitate the removal of coronoid. The same procedure is carried bilaterally if needed.

The mean preoperative inter-incisal opening of 14.40 mm in OSMF patients has been reported to increase the mouth opening to 24.60 mm with fibrotomy alone and to 35–44.80 mm after unilateral and bilateral coronoidectomy, respectively [37].

Reconstruction of Surgical Defect

In order to avoid contracture due to scarring after fibrotomy, it is always recommended to reconstruct the surgical defect. Various options of flaps are available for the coverage of defect so produced:

- Buccal fat pad
- Nasolabial flap
- Superficial temporal fascia flap
- Tongue flap
- Split skin graft
- Dermal fat graft

- Collagen graft
- Radial forearm flap

Buccal Fat Pad

Heister [38] was the first to identify buccal fat pad (BFP) and believed it to be glandular in nature and so called it "glandula molaris". In 1801, Bichat described BFP as a fatty tissue. Its use increased after Egyedi described methods of using BFP as a versatile pedicle graft for closure of oronasal and oroantral communications as well as postsurgical maxillary defects [39]. Tideman detailed its anatomy, vascular supply, and surgical technique. It offers the advantage of a rich blood supply to promote healing. The grafted fat pad closes the dead space area, promotes granulation, and limits scar contraction. Also, BFP offers strong anti-infective and reconstructive advantages [40] for medium-sized (5 × 4 cm) defects in the buccal mucosa.

An incision is made over oral mucosa adjacent to the maxillary vestibule in the region of the second and third molars, preventing damage to the parotid duct (Fig. 8.4). Blunt dissection through the buccinator muscle leads to the body or its buccal extension, which is gently teased into the defect, taking care not to rupture its delicate capsule. Adequate volume of BFP ensures tension-free closure using Vicryl sutures at the periphery of the graft.

Nasolabial Flap

The nasolabial flap is a pedicled, elliptical transposition flap in the nasolabial fold region supplied by the angular artery. It serves to reconstruct small- to medium-size defects in the oral cavity with minimal aesthetic consequences. The rich blood supply allows it to even cover large defects with cosmetic results [41].

It is designed in sufficient length and width to fill the entire defect without tension. Incision is limited to the subdermal tissues to leave the base of flap intact.

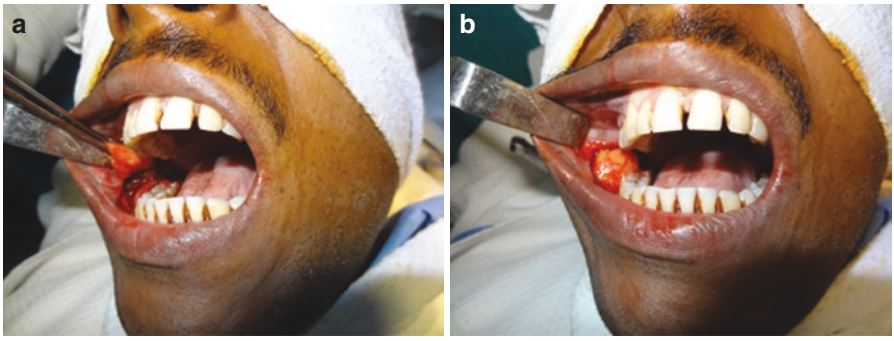


Fig 8.4 (**a**) Buccal fat pad graft pedicled. (**b**) Positioned to cover the surgical defect

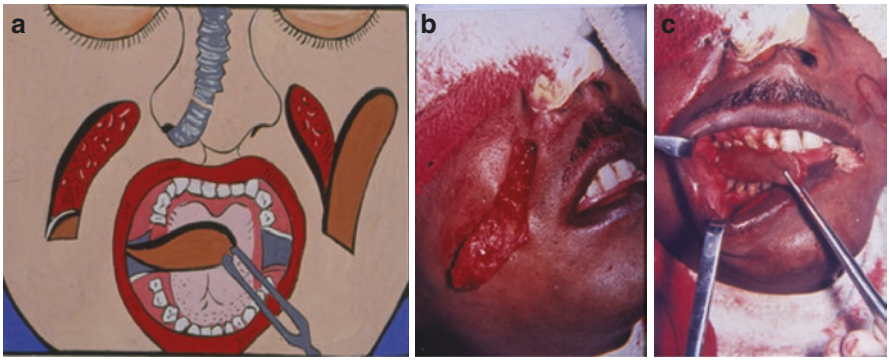


Fig 8.5 (**a**) Nasolabial flap design. (**b**) Incision made. (**c**) Nasolabial flap rotated

Extended nasolabial flaps reach up to the inferior border of the mandible and are raised bilaterally, taking care not to disturb the facial muscles [42]. A tunnel made near the base of the flap facilitates its entry in the oral cavity (Fig. 8.5). De-epithelialization and rotation of the flap in the oral cavity allow closure of the defect and are sutured with 3-0 black silk. The skin is undermined to facilitate its subcuticular closure using 4-0 Prolene. Pressure dressing using antibiotic-soaked sterile gauze or betadine-soaked gauze is placed over the graft intraorally to keep it in contact with mucosal defect. Sutures are removed after 10 days of surgery [43].

Superficial Temporal Fascia Flap

A preauricular incision in the hair-bearing area is extended into the temporal region. Further dissection allows the development of the superficial temporal fascia flap (Fig. 8.6). The superficial temporal fascia flap is passed below the zygomatic arch to be brought intraorally and sutured. This procedure can be performed bilaterally to bring in good blood supply to the fibroses area and improve the clinical result [44].

Tongue Flap

The pedicled tongue flap is used for covering oral defects as a two-stage procedure. Either the anterior two third or posterior third of the tongue can be used for the tongue flap. After around 21 days, the tongue pedicle is divided from its base. All third molars are extracted to prevent trauma to the tongue flap.

The tongue is infiltrated with local anaesthesia solution containing adrenaline (epinephrine). On the dorsolateral aspect of the tongue, an incision is made parallel to the midline of the tongue up to 1 cm behind the tip of the tongue (Fig. 8.7). On the lateral margin of the tongue, it is taken down inferiorly up to the last molar. Deep elliptical tongue flap is raised and rotated outward laterally to cover the raw area in the cheek and sutured. The wound in the tongue is primarily closed. Similar procedure is undertaken on the contralateral side.

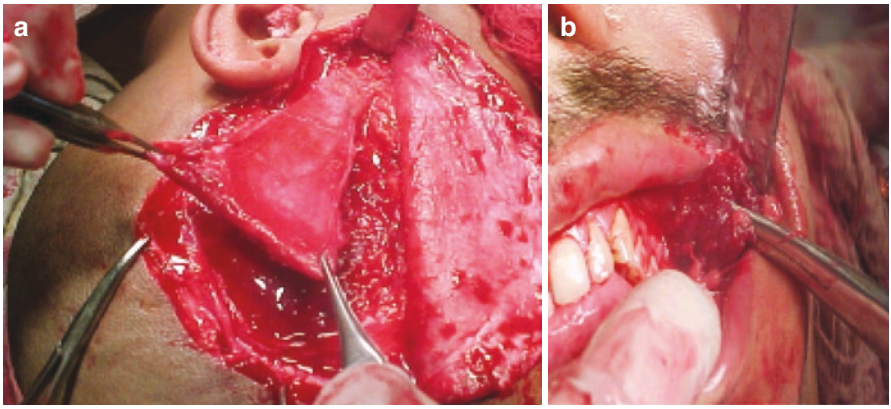


Fig 8.6 (**a**) Superficial temporal flap raised (**b**) Position intraoral

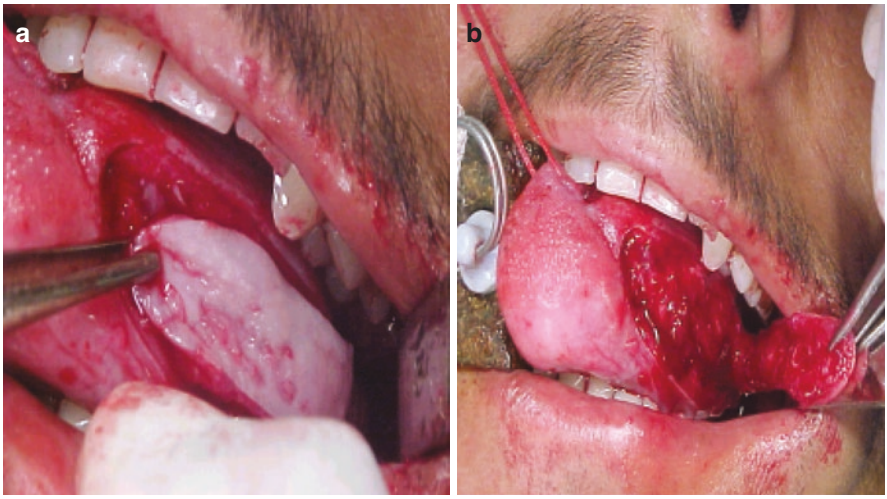


Fig 8.7 (**a**) Tongue flap incision. (**b**) Tongue flap raised

This flap has a high success rate due to its viability and resistance to OSMF. The postoperative mobility of the tongue and articulation become normal in about 4–6 weeks, and dullness of taste sensation is negligible [45]. The reduction in the bulk of the tongue is regained with tongue movements and exercises.

Split Skin Graft

Split skin graft can be easily harvested from the thigh laterally using Humby's knife or approximately 0.014–0.018-in. thick surgical dermatome (Fig. 8.8). Donor site is dressed with antibiotic-impregnated gauze, while the harvested graft is placed on a

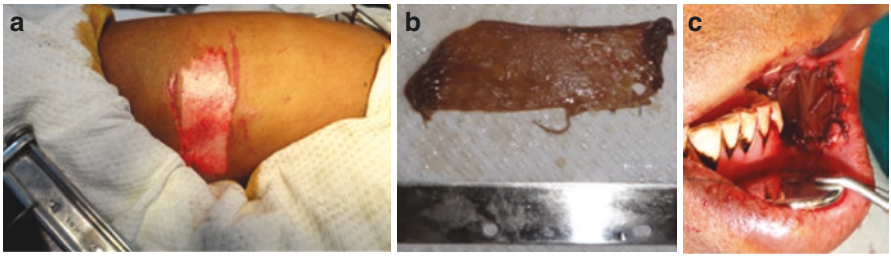


Fig 8.8 (**a**) Split skin harvesting. (**b**) Split skin graft. (**c**) Sutured onto defect

wet wooden slab to be cut into desired size and shape [45]. Puncture holes are made before suturing it to the mucosal margins. A trans-buccal quilt suture is important to minimize the dead space and maintain its close contact with the wound bed. A sterile antibiotic-soaked gauze is placed intraorally to cover the graft for the next few days.

Dermal Fat

An elliptical incision is made supero-medial to iliac crest in the abdomen, and the epidermis is dissected finely and discarded. The underlying dermis and subcutaneous fat are dissected as a dermis-fat graft and sutured intraorally, such that the dermal layer faces the oral cavity (Fig. 8.9).

Collagen Sheath

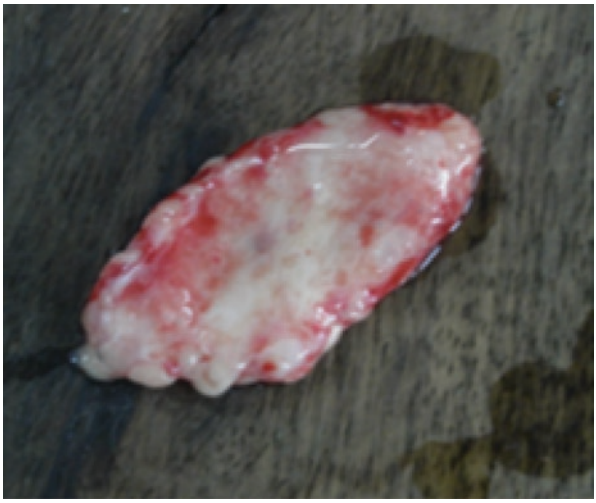
A commercially available collagen sheath may be used as a dressing to cover the surgical defect and sutured to the surrounding mucosa on its edges. A pompom dressing holds the sheath closely adapted to the wound bed. It is shown to allow a faster epithelialization (Fig. 8.10).

Radial Forearm Flap

A bipaddled radial forearm flap can be obtained with at least 4 cm pedicle for microvascular anastomosis to the nearest facial artery and vein. This surgery is simultaneously performed by two teams. The proximal flap is designed to cover the ipsilateral buccal mucosal defect, while the distal flap covers the contralateral buccal defect. The length of the "bridge pedicle" between these two flaps should always be 8–10 cm [46].

Postoperatively, these patients are kept on nasogastric feeding for 10 days [47]. Mouth-opening exercises are started on the fifth postoperative day and continued for 6 months at least.

Fig 8.9 Dermal fat graft



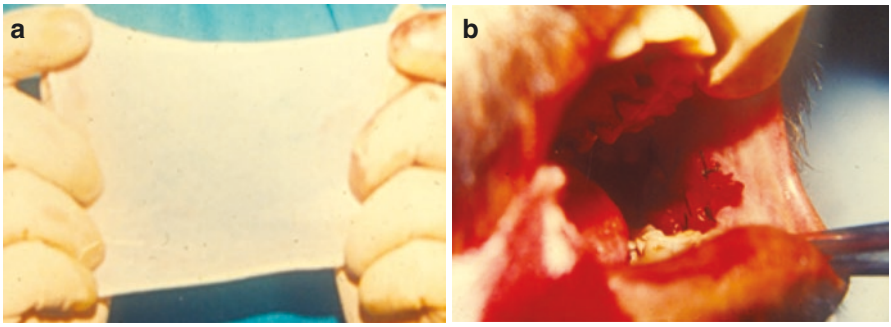


Fig 8.10 (**a**) Collagen sheath. (**b**) Collagen sheath in position

Physiotherapy

Oral physiotherapy supports the surgical treatment of OSMF and helps in improving the range of mouth opening and preventing relapse. Physiotherapy includes forceful mouth opening, heat, or ultrasound massage therapy.

Massage therapy improves the elasticity of fibrous tissues and mobilizes scar tissues. This gentle tissue manipulation improves cheek, tongue, and lip extensibility.

Muscle stretching exercises for the mouth prevent further restriction of mouth movements and relapse. This can be performed by using mouth gag and acrylic surgical stent, ballooning of the mouth, whistling, lip exercising, hot water gargling, and gradually increasing the number of inter-positioned spatula between the teeth. It is recommended to exercise at least for 6 months to get the result.

Heat-shortwave/microwave diathermy: Lukewarm water, hot rinses, or selective deep heating therapies like shortwave or microwave diathermy improve the

suppleness of the oral mucosa, through separation of collagen fibres and softening of the cementing substance. Microwave diathermy is superior to shortwave, as it allows selective heating of the juxta-epithelial connective tissue. Microwave diathermy at 2450 MC/s daily for 20 min at each site, with 20–25 W of energy, 15 sittings, can give good results in moderately advanced but poor in very advanced cases.

Ultrasound: Ultrasound allows increased cell membrane permeability by altering sodium and potassium ion gradients. This increases vasodilatation, accelerates lymph flow, decreases inflammation, and stimulates metabolism. Ultrasound over the cheek for 15 consecutive days, using a 5 cm diameter transducer head for 3–4 min to each side at a frequency of 3 MHz and intensity of 0.5–3 w/sqcm, accelerates healing, increases the extensibility of collagen fibres, and provides pain relief by selectively raising the temperature.

References

1. Li N, Jian X, Hu Y, Xu C, Yao Z, Zhong X. Discovery of novel biomarkers in oral submucous fibrosis by microarray analysis. Cancer Epidemiol Biomarkers Prev. 2008;17(9):2249–59.
2. Arakeri G, Brennan PA. Oral submucous fibrosis: an overview of the aetiology, pathogenesis, classification, and principles of management. Br J Oral Maxillofac Surg. 2013;51(7):587–93.
3. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36(10):575–80.
4. Schwartz J. Atrophia Idopathica mucosa oris. In: Proceedings of the 11th International Dental Congress; 1952. London, UK.
5. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol. 1966;22(6):764–79.
6. Tilakaratne WM, Ekanayaka RP, Warnakulasuriya S. Oral submucous fibrosis: a historical perspective and a review on aetiology and pathogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):178–91.
7. Ranganathan K, Uma Devi M, Joshua E, Kirankumar K, Saraswathi TR. Oral submucous fibrosis: a case control study in Chennai South India. J Oral Pathol Med. 2004;33:274–7.
8. Gupta MK, Mhaske S, Ragavendra R. Imtiyaz. Oral submucous fibrosis - current concepts in etiopathogenesis. People J Sci Res. 2008;1:39–44.
9. Aziz SR. Oral submucous fibrosis: case report and review of diagnosis and treatment. J Oral Maxillofac Surg. 2008;66:2386–9.
10. Zhang SS, Li WH, Gao YJ, Liu ZW, Liu L, Tang JQ, et al. Betel-quid and oral submucous fibrosis: a cross-sectional study in Hunan province, China. J Oral Pathol Med. 2012;41:748–54.
11. Reichart PA, Nguyen XH. Betel quid chewing, oral cancer and other oral mucosal diseases in Vietnam: a review. J Oral Pathol Med. 2008;37:511–4.
12. Yen AM, Chen SL, Chiu SY, Chen HH. Association between metabolic syndrome and oral pre-malignancy: a community and population-based study (KCIS No. 28). Oral Oncol. 2011;47:625–30.
13. Mehrotra D, Kumar S, Mishra S, Kumar S, Mathur P, Pandey CM, Pandey A, Chaudhry K. Pan masala habits and risk of oral precancer: a cross-sectional survey in 0.45 million people of North India. J Oral Biol Craniofac Res. 2017;7(1):13–8.
14. Yang S-F, et al. Changes in prevalence of precancerous oral submucous fibrosis from 1996 to 2013 in Taiwan: a nationwide population-based retrospective study. J Formosan Med Assoc. 2018;117:147. https://doi.org/10.1016/j.jfma.2017.01.012.

15. Paymaster JC. Cancer of the buccal mucosa; a clinical study of 650 cases in Indian patients. Cancer. 1956;9(3):431–5.
16. Aziz SR. Coming to America: betel nut and oral submucous fibrosis. JADA. 2010;141:423–8.
17. Ho MWS. Patients with both oral leukoplakia and oral submucous fibrosis had a higher rate of malignant transformation compared to patients with oral leukoplakia or oral submucous fibrosis alone. J Evid Based Dent Pract. 2013;13(4):183–4.
18. Shiau YY, Kwan HW. Submucous fibrosis in Taiwan. Oral Surg Oral Med Oral Pathol. 1979;47(5):453–7.
19. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis, and future research. Bull World Health Organ. 1994;72(6):985–96.
20. Mehrotra D, Kumar S, Agarwal GG, Asthana A, Kumar S. Odds ratio of risk factors for oral submucous fibrosis in a case control model. Br J Oral Maxillofac Surg. 2013;51:e169–73.
21. Arakeri G, Hunasgi S, Colbert S, Merkx MAW, Brennan P. Role of drinking water copper in pathogenesis of oral submucous fibrosis: a prospective case control study. Br J Oral Maxillofac Surg. 2014;52(6):507–12.
22. Arakeri G, Colbert S, Patil SG, Hale B, Merkx MAW, Brennan P. Salivary pooling: is it specific to particular regions in oral submucous fibrosis. Br J Oral Maxillofac Surg. 2015;53(3):275–8.
23. Isaac U, Isaac JS, Khoso NA. Histopathologic features of oral submucous fibrosis: a study of 35 biopsy specimens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;10:556–60.
24. Pindborg JJ. Oral submucous fibrosis: a review. Ann Acad Med Singapore. 1989;18(5):603–7. Review.
25. Mehrotra D, Prandhan R, Gupta S. Retrospective comparison of surgical treatment modalities in 100 patients with OSMF. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107:e1–10.
26. Khanna JN, Andrade NN. Oral submucous fibrosis: a new concept in surgical management. Report of 100 cases. Int J Oral Maxillofac Surg. 1995;24(6):433–9.
27. Shevale VV, Kalra RD, Shevale VV, Shringarpure MD. Management of oral sub-mucous fibrosis: a review. Ind J Dent Sci. 2012;4:107–14.
28. Dayanarayana U, Doggalli N, Patil K, et al. Non surgical approaches in treatment of OSF. J Dent Med Sci. 2014;13(11):63–9.
29. Lin HJ, Lin JC. Treatment of oral submucous fibrosis by collagenase: effects on oral opening and eating function. Oral Dis. 2007;13(4):407–13.
30. Haque MF, Meghji S, Nazir R, Harris M. Interferon gamma (IFN-gamma) may reverse oral submucous fibrosis. J Oral Pathol Med. 2001;30(1):12–21.
31. Tai YS, Liu BY, Wang JT, Sun A, Kwan HW, Chiang CP. Oral administration of milk from cows immunized with human intestinal bacteria leads to significant improvements of symptoms and signs in patients with oral submucous fibrosis. J Oral Pathol Med. 2001;30:618–25.
32. Sankaranarayanan S, Padmanaban J, Ramachandran CR, Manjunath S, Baskar S, Senthil Kumar R, Senthil Nagarajan R, Murugan P, Srinivasan V, Abraham S. Autologous bone marrow stem cells for treatment of oral sub-mucous fibrosis - a case report. Sixth Annual Meeting of International Society for Stem Cell Research (ISSCR), Philadelphia, PA USA. 2008.
33. Nagpal M, Sood S. Role of curcumin in systemic and oral health: an overview. J Nat Sci Biol Med. 2013;4(1):3–7.
34. Patil S, Santosh BS, Maheshwari S, Deoghare A, Chhugani S, Rajesh PR. Efficacy of oxitard capsules in the treatment of oral submucous fibrosis. J Can Res Ther. 2015;11(2):291–4.
35. Hastak K, Lubri N, Jakhi SD. Effect of turmeric oil and turmeric oleoresin on cytogenic damage in patients suffering from oral submucous fibrosis. Cancer Lett. 1997;116:265–9.
36. Mulk BS, Deshpande P, Velpula N, Chappidi V, Chintamaneni RL, Goyal S. Spirulina and pentoxifylline - a novel approach for treatment of oral submucous fibrosis. J Clin Diagn Res. 2013;7(12):3048–50.
37. Gupta H, Tandon P, Kumar D, Sinha VP, Gupta S, Mehra H, Singh J. Role of coronoidectomy in increasing mouth opening. Natl J Maxillofac Surg. 2014;5(1):23–30.
38. Heister L. Compendium anatomicum. In: Weberi GC. Norimbergac, Germany; 1732. p. 146.

39. Jain MK, Ramesh C, Sankar K, Lokesh Babu KT. Pedicled buccal fat pad in the management of oroantral fistula: a clinical study of 15 cases. Int J Oral Maxillofac Surg. 2012;41:1025–9.
40. Rai A, Datarkar A, Rai M. Is buccal fat pad a better option than nasolabial flap for reconstruction of intraoral defects after surgical release of fibrous bands in patients with oral submucous fibrosis? A pilot study: a protocol for the management of oral submucous fibrosis. J Craniomaxillofac Surg. 2014;42(5):e111–6.
41. Borle RM, Nimonkar PV, Rajan R. Extended nasolabial flaps in the management of oral submucous fibrosis. Br J Oral Maxillofac Surg. 2009;47(5):382–5.
42. Agrawal D, Pathak R, Newaskar V, Idrees F, Waskle R. A comparative clinical evaluation of the buccal fat pad and extended nasolabial flap in the reconstruction of the surgical defect in oral submucous fibrosis patients. J Oral Maxillofac Surg. 2018;76:676.e1. https://doi.org/10.1016/j.joms.2017.11.013. ISSN: 0278-2391.
43. Kothari MC, Hallur N, Sikkerimath B, Gudi S, Kothari CR. Coronoidectomy, masticatory myotomy and buccal fat pad graft in management of advanced oral submucous fibrosis. Int J Oral Maxillofac Surg. 2012;41(11):1416–21.
44. Ramadass T, Manokaran G, Pushpala SM, Narayanan N, Kulkarni GN. Oral submucous fibrosis - new dimensions in surgery. Ind J Otolaryngol Head Neck Surg. 2005;57(2):99–102.
45. Mehrotra D, Pradhan R, Mohammad S, Jaiswara C. Random control trial of dermis-fat graft and interposition of temporalis fascia in the management of temporomandibular ankylosis in children. Br J Oral Maxillofac Surg. 2008;46:521–6.
46. Lee JT, Cheng LF, Chen PR, Wang CH, Hsu H, Chien SH, Wei FC. Bipaddled radial forearm flap for the reconstruction of bilateral buccal defects in oral submucous fibrosis. Int J Oral Maxillofac Surg. 2007;36(7):615–9.
47. Warnakulasuriya S, Kerr AR. Oral submucous fibrosis: a review of the current management and possible directions for novel therapies. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):232–41.

Chapter 9
Clinical Presentation of Oral Mucosal Premalignant Lesions

Michaela Goodson

This chapter is concerned with the clinical presentation of premalignant oral mucosal lesions and identification of lesions as high or low risk for malignant transformation. In 2005, the WHO renamed the premalignant lesions as "oral potentially malignant disorders", a term that suggests malignant transformation may not be an inevitable consequence, rather a possibility, and may occur at a site distinct from the original presenting lesion. Evidence suggests that for dysplastic oral potentially malignant lesions, approximately 40% change very little with time, 20% can regress spontaneously and a further 20% may increase in size. Overall, 20% are at risk of malignant transformation. Unfortunately, there are currenty no highly sensitive or specific biomarkers available that can accurately predict malignant transformation. Management decisions are therefore decided by evaluating individual patients' risk in relation to known clinical and pathological risk factors. Accurately describing the clinical appearance of lesions is critical for communication with colleagues and for individual clinicians to follow up patients longitudinally.

In order to describe the clinical presentation of potentially malignant disorders, it is necessary to briefly explain the historical and current nomenclature used in clinics worldwide. Much of the difficulty in concluding optimal management strategies for patients with oral potentially malignant disorders in systematic reviews and meta-analysis of studies on oral potentially malignant disorder outcomes has arisen from differences used in the nomenclature of lesions.

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Nomenclature of Potentially Malignant Disorders

In 1972, the WHO distinguished a precancerous lesion as "a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart". In contrast, a precancerous condition is defined as a "generalised state associated with a significantly increased risk of cancer" [1, 2].

In the late 1970s, precancerous lesions included leukoplakia (Fig. 9.1), erythroplakia and palatal lesions in reverse smokers, whereas precancerous conditions included submucous fibrosis, actinic keratosis, lichen planus and discoid lupus erythematosus. There was however confusion surrounding this terminology because it did not accurately predict which lesions or conditions were high or low risk.

In 2005, the WHO Collaborating Centre for oral cancer and precancer came up with consensus views on working terminology to help with the classification of lesions of the oral mucosa based on the following observations:

1. Longitudinal studies showed that some areas of the oral mucosal tissue with alterations in clinical appearance underwent malignant change at follow-up.
2. The coexistence of red and white areas at the peripheral margins of squamous cell carcinoma suggests that squamous cell carcinoma may have a precursive state.
3. Some lesions show evidence of dysplasia (morphological and cytological changes without invasion of the basement membrane), but are not frankly invasive so may represent a precursive state.
4. Chromosomal, genomic and molecular alterations found in oral squamous cell carcinomas have also been found in oral mucosal precursor lesions.

In 2007, the WHO identified the term "potentially malignant disorders" to denote what had previously been termed precancer, precursor lesions, premalignant intraepithelial neoplasia or potentially malignant lesions. It was also suggested that precancerous conditions and lesions should not be subdivided because the subdivision gave little indication of the risk of malignant transformation. Given that patients with single precancerous lesions can develop cancerous lesions at contralateral previously healthy sites and molecular aberrations can occur at sites of normal clinical appearance, there appears to be little prognostic value in labelling patients as having oral precancerous lesions or conditions [3, 4].

Potentially malignant disorders therefore include patients with leukoplakia, proliferative verrucous leukoplakia (PVL), erythroplakia, palatal lesions in reverse smokers, chronic hyperplastic candidosis, sideropenic dysphagia, oral submucous fibrosis, actinic keratosis, lichen planus, discoid lupus erythematosus and hereditary disorders that have an increased risk of malignancy in the mouth including dyskeratosis congenita and epidermolysis bullosa. Within the potentially malignant disorders is a spectrum of disease encompassing single discrete lesions to multiple lesion disease which may or may not be associated with a systemic disorder.

Assessment of the Patient with Potentially Malignant Disorders

In general, the majority of patients present with lesions to their general dental practitioner with the presenting complaint of a red or white patch of the oral mucosa. A minority of patients present to a general medical practitioner. In the Western world, patients are mostly subsequently referred to a specialist clinic for management in a secondary or tertiary referral centre.

Evaluating the Patient with Oral Potentially Malignant Disorders (OPMDs)

Patients commonly present with incidental painless white or red patches of the oral mucosa found at routine dental check-up appointments. A minority of patients will present with painful red patches or nonhomogeneous white patches for which they have used various home or over-the-counter remedies to alleviate the lesion.

When taking a history from a patient with a new suspected oral potentially malignant disorder, it is important to try and assess whether the patient indeed has an oral potentially malignant disorder rather than a reversible lesion that could be due to tooth trauma, for example. There is therefore a need to assess the size and site of the lesion; the length of time a lesion has been present; previous lesions that have been treated and resolved; any changes in colour, shape and size; or surface characteristics of a new lesion in recent weeks or months. Patients should also be questioned as to whether they remember the onset of the lesion coinciding with tooth trauma event or use of topical medication for another mucosal condition. An assessment of the patient at the first visit aims to diagnose patients at high risk for having an oral potentially malignant disorder or a high risk for malignancy as this will determine management strategies for each individual patient.

Within the medical history, it is important to ascertain whether the patient is immunocompromised or has been so in the past as this could pose a higher risk for a patient developing a OPMD or squamous carcinoma. The drug history may also suggest medications that could be responsible for reversible mucosal ulceration and lesions that mimic oral potentially malignant disorders (e.g. nicorandil ulceration of the oral mucosa). The drug history may also highlight immunocompromising medications that could accelerate the development of OPMDs and squamous cell carcinoma (SCC).

An allergy history will inform the clinician if the lesion in their mouth could be reversible (e.g. amalgam related) if the allergen is avoided, reducing the need for biopsies and further investigation.

The social history is important to evaluate the amount and type of tobacco use, preferential site of placement and historical use of tobacco products because refrain-

ing from the use of these products may prevent development of SCC or even reverse a OPMD. An alcohol history evaluating units and type of alcohol consumed with or without concurrent tobacco use is useful to evaluate whether the patient is at higher risk of OPMD development or rapid progression to SCC. Diet, fruit and vegetable consumption has been shown to influence the development of OPMDs in a number of studies particularly in conjunction with alcohol drinking [5, 6]. Poor oral health and hygiene has been shown to be an independent risk factor for oral cancer in China, the USA and Brazil [7–10], but poor oral health and hygiene is often found in individuals with multiple confounding risk factors, so the exact influence of oral health as an independent variable is unknown.

The family history for a patient with a OPMD may give some indication of high risk for inherited disorders that might give an increased risk for malignancy, e.g. epidermolysis bullosa, or identify a benign condition that could be confused for a OPMD, e.g. white sponge naevus with autosomal dominant inheritance present in a direct relative.

Adjunctive diagnostic aids such as VELscope® or methylene blue may also be used during the clinical examination, but their lack of specificity and sensitivity in comparison to the gold standard of clinical examination means they are of limited use in assessing a new clinical presentation.

In [11], Goodson and Thomson reported an evaluation of the VELscope in identifying high-risk patients who might benefit from interventional surgical treatment of OPMDs. In this study, 296 patients were evaluated to see whether VELscope could improve diagnostic accuracy. The study found that there was a marginal improvement in diagnostic accuracy as far as assessing the extent of a lesion for biopsy, but VELscope was not very sensitive at identifying worsening dysplasia grades and could not differentiate inflammation consistently from dysplasia. In conclusion, it was stated that at best, VELscope could be used as a clinical adjunct to aid examination, but could not replace the gold standard of standard clinical examination.

A photograph of the lesion(s) however may be useful as they can be used as a record to assess the change of lesions at subsequent appointments.

Clinical Presentation of Oral Potentially Malignant Disorders

Potentially malignant disorders are commonly described by clinicians as leukoplakia (homogeneous or nonhomogeneous) or erythroplakia.

Leukoplakia

Leukoplakia is the most common clinical presentation of an oral potentially malignant disorder comprising around 60–70% oral potentially malignant disorders with prevalence rates of 3–105 cases per 1000 population worldwide. Higher prevalence

rates are found in New Guinea, China and India, and in general, rates are higher in rural than urban populations.

Leukoplakia can be defined as "plaques of questionable risk having excluded (other) known diseases or disorders that carry increased risk for cancer". Leukoplakia is purely a clinical condition, and there are no histological requirements for a lesion to fulfil this definition.

Leukoplakia can exist in a homogeneous or nonhomogeneous form where homogeneous types are purely white patches and nonhomogeneous are speckled, nodular or verrucous lesions with speckled nonhomogeneous lesions being those at greatest risk of malignant transformation.

Leukoplakia on standard clinical examination appears white because keratin in the lesion absorbs water from saliva giving a white appearance. As a consequence, it is not until the lesions are around 1 cm in size or more that they are recognised by patients or the general practitioner as visible mucosal keratinisation and thickening. Diagnostic aids such as methylene blue and VELscope may help to diagnose smaller or less visible lesions, but the prognostic value of these diagnostic adjuncts is uncertain.

The risk factors for development of leukoplakia include tobacco use, alcohol consumption, dietary factors, oral health, immune suppression and low socioeconomic status.

The sites of clinical presentation may therefore be influenced by these factors. Tobacco can be used in smoked or smokeless forms, and the site of leukoplakia may be related to habitual placement of the tobacco product. Clinically, a lesion may start as an area of wrinkled slightly whitened mucosa often in the floor of the mouth or ventral surface of the tongue but progress through a thickened smooth plaque to an irregular thickened plaque (sometimes described as verrucous) which may show yellow or blackened discolouration from tobacco products (Figs. 9.1 and 9.2).

Leukoplakia has an overall prevalence of around 2–3% but is more common in males and is most common in the 50–70-year-old age group. There is however evidence in Western populations of an increased number of younger patients presenting with leukoplakia and subsequent malignant transformation in the absence of common risk factors.

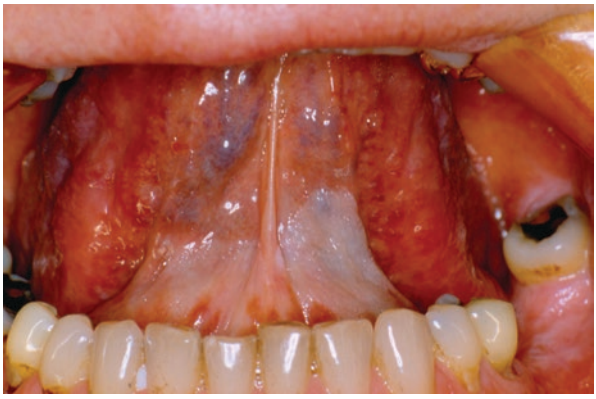

Fig. 9.1 Early faint leukoplakia of the floor of the mouth (@ John Wiley & Sons, reproduced with permission)

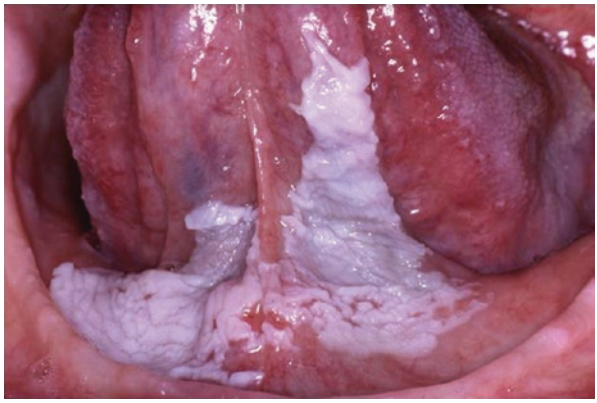

Fig. 9.2 Thickened irregular leukoplakia of the floor of the mouth (@ John Wiley & Sons, reproduced with permission)

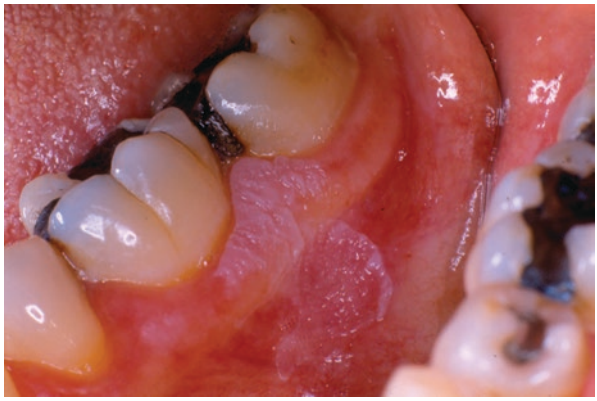

Fig. 9.3 Early localised proliferative verrucous leukoplakia of the buccal gingivae and left buccal sulcus (@ John Wiley & Sons, reproduced with permission)

Proliferative Verrucous Leukoplakia

Proliferative verrucous leukoplakia (Figs. 9.3 and 9.4) is a clinical entity that appears to behave more aggressively than erythroplakia with malignant transformation rates of 60–100% [12–14]. Proliferative verrucous leukoplakia differs from leukoplakia in general in the way it progresses from a flat lesion through increasing degrees of thickening, fissuring and warty proliferation until the eventual development of squamous cell carcinoma. Even if lesions are surgically removed, they have a high chance of recurrence and new lesion development [15]. PVL commonly presents with multiple lesions involving more than one site. Signs suggesting malignant transformation are new areas of redness or erosions within a lesion, induration and rapid growth of a verrucous patch.

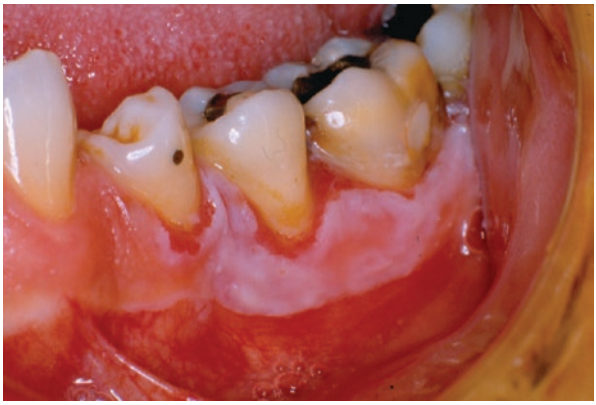

Fig. 9.4 More extensive proliferative verrucous leukoplakia of the left buccal gingivae and buccal sulcus (@ John Wiley & Sons, reproduced with permission)

Risk Factors for Leukoplakia and Erythroplakia

Tobacco Use

Tobacco use and smoking are common risk factors. The type of tobacco used usually determines the site of the leukoplakia lesion. Leukoplakia is around six times more common in smokers than non-smokers and can affect any part of the oral mucosa, although the buccal mucosa, floor of the mouth and ventrolateral tongue are commonly affected. Some patients however use smokeless forms of tobacco. Snuff, tobacco chewing and use of tobacco products mixed with areca nut and lime/additives can result in different site presentations depending on the site where the tobacco is usually placed on the oral mucosa.

Alcohol Consumption

Long-term alcohol use has also been cited as a risk factor for leukoplakia. For patients with dysplastic oral premalignant lesions that have been excised, there is evidence that continued alcohol intake of more than 28 units per week increases the risk of recurrent disease at the same site [16].

Immune Compromise

Immune suppression has long been known to be a risk factor for oral cancer, but evidence supporting a role in precancer is largely anecdotal and related to case reports of leukoplakia in transplant patients and patient with HIV/AIDS. In HIV/

AIDS patients, dormant Epstein-Barr virus can be reactivated when the immune system is weakened resulting in a condition called hairy leukoplakia.

Socioeconomic status has been found to be an independent predictor of oral leukoplakia in the US Third National Health and Nutrition Examination Survey (NHANES III) along with diabetes, age and tobacco smoking. Alcohol use, race/ethnicity, years of education and BMI however showed no independent effects. There is however no evidence that socioeconomic status of a patient can predict the site of a lesion [17].

Diet

The influence of dietary factors on precancerous lesions has been assessed in India [5] where low intakes of iron and vitamin C in women were associated with the presence of leukoplakia and precancer. Again, the site of presentation of leukoplakia was not influenced by these variables. In erythroplakia, a case-control study of 100 cases found that there was a multiplicative effect between alcohol consumption and low vegetable intake or low fruit intake [6].

Oral Health

There is no evidence that oral health and hygiene is an independent predictor of oral precancer development at the present time although poor oral hygiene may coexist in patients with many of the other precancer risk factors previously described.

Human Papilloma Virus

There is conflicting evidence of HPV-16 and 18 in oral potentially malignant disorder development. Observational studies suggest it is present in up to 20% of lichen planus lesions which could be described clinically as leukoplakia. Chen et al. [18] found that HPV-18 was a significant risk factor for leukoplakia and squamous papilloma and the site of leukoplakia was more often in the oropharynx.

The ARCAGE study in 2013 [19] looked at more than 1400 cases and controls with upper aerodigestive tract cancers and found an important role for HPV-16 infection in oropharyngeal cancer and supported a marginal role for HPV-18 in oropharyngeal cancer and HPV-6 in laryngeal cancer, but not oral cancer specifically.

Erythroplakia

Erythroplakia (Fig. 9.5) is less commonly seen than leukoplakia and has a reported prevalence of 0.2–1.9 per 1000 population from studies in the USA and Mexico [20, 21]. It is defined as "a fiery red patch that cannot be characterized clinically or pathologically as any other definable disease". It is more common to see patients with erythroleukoplakia—a lesion that is a combination of red and white in appearance, sometimes termed speckled leukoplakia (Fig. 9.6). Again, erythroplakia is a clinical diagnosis, and the term gives no indication of histological findings [22].

Erythroplakia and erythroleukoplakias have greater potential for malignant transformation than homogeneous leukoplakia, so identification of red areas within a lesion is important clinically (Table 9.1).

Erythroplakias are considered to be very high-risk lesions for malignant transformation. They are more commonly symptomatic lesion, presenting with soreness or sensitivity. Erythroplakia is, again, purely a clinical diagnosis, presenting with almost equal prevalence in men and women. Erythroplakia is red in colour because lesions commonly have atrophic epithelium and histologically may demonstrate dysplasia or even carcinoma in situ. It is not uncommon to find early carcinoma in erythroplakic lesions.

Erythroleukoplakia or speckled leukoplakia presents as leukoplakia on a background of erythroplakia most commonly found at the labial commissures or the floor of the mouth. It is often superimposed with chronic candidal infection.

The Differential Diagnosis of Leukoplakia and Erythroplakia

There are a number of benign white and red lesions that can be confused with leukoplakia and erythroplakia.

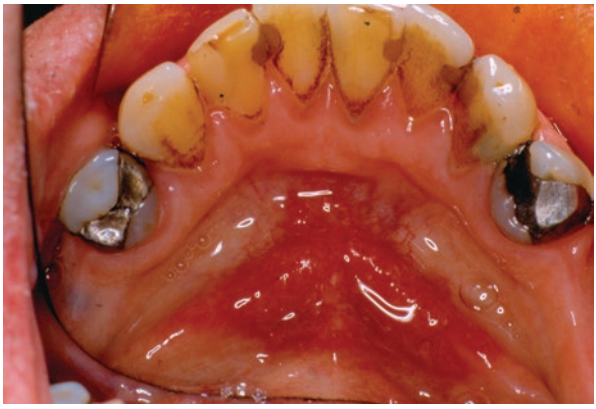

Fig. 9.5 Erythroplakia of the floor of the mouth (@ John Wiley & Sons, reproduced with permission)

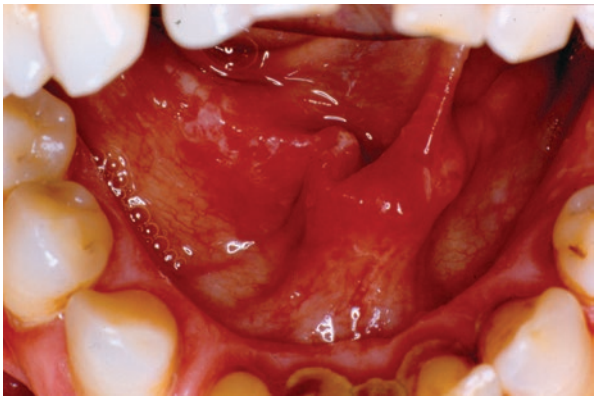

Fig. 9.6 Erythroleukoplakia of the floor of the mouth (@ John Wiley & Sons, reproduced with permission)

Table 9.1 Worldwide prevalence rates of oral potentially malignant disorders per 1000 population

Lesion	Prevalence rate per 1000
Leukoplakia	4–105
Erythroplakia	0.2–1.9

The differential diagnosis of leukoplakia could be one of a number of hereditary conditions (including oral epithelial white sponge naevus, leukoedema, pachyonychia congenita, tylosis, follicular keratosis, hereditary benign intraepithelial dyskaryosis) or chronic inflammations (oral lichen planus, frictional keratosis, chemical or thermal trauma).

The differential diagnosis of erythroplakia includes inflammatory disorders such as desquamative gingivitis, erosive lichenoid lesions, pemphigoid, and hypersensitivity reactions; infections including candidosis, purpura and trauma; or tumours such as Kaposi's sarcoma and haemangioma.

Premalignant Lesions Versus Premalignant Conditions

Premalignant lesions (leukoplakia, erythroplakia and erythroleukoplakia) present as single isolated lesions of morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart (WHO, 1978), but there are a number of more generalised medical conditions which may result in the development of potentially malignant lesions. In contrast to oral precancerous lesions where a discrete mucosal lesion may present, these more generalised states are associated with a significantly increased risk of cancer, and this includes a range of systemic disorders where the oral manifestations are one of the many signs or symptoms of disease. The oral precancerous conditions include immune suppression, lichen planus (Fig. 9.7) and lichenoid lesions, oral submucous fibrosis (Fig. 9.8), sideropenic

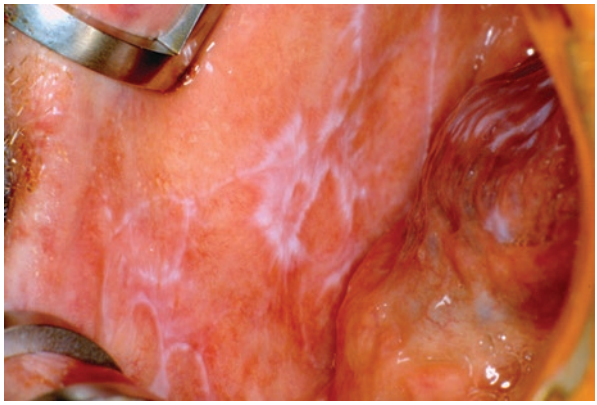

Fig. 9.7 Reticular lichen planus of the buccal mucosa (@ John Wiley & Sons, reproduced with permission)

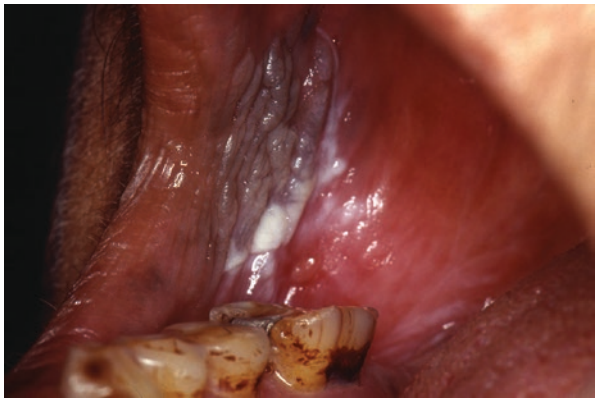

Fig. 9.8 Oral submucous fibrosis of the right buccal mucosa (@ John Wiley & Sons, reproduced with permission)

dysphagia, discoid lupus erythematosus, actinic cheilitis (Fig. 9.9), syphilis (Fig. 9.10) and dyskeratosis congenita.

Immunosuppression

Drug-induced immunosuppression which may occur following organ transplant is more common than congenital immune suppression, but acquired immune deficiency in HIV/AIDS, for example, is increasingly common worldwide. It is common for immunosuppressed patients to present with multiple oral leukoplakias with the lips and labial commissure as common sites. These lesions may occur synchronously or at different times. Immunosuppressed patients are at high risk of developing squamous cell carcinoma and as such need close monitoring for progression or recurrence of disease.

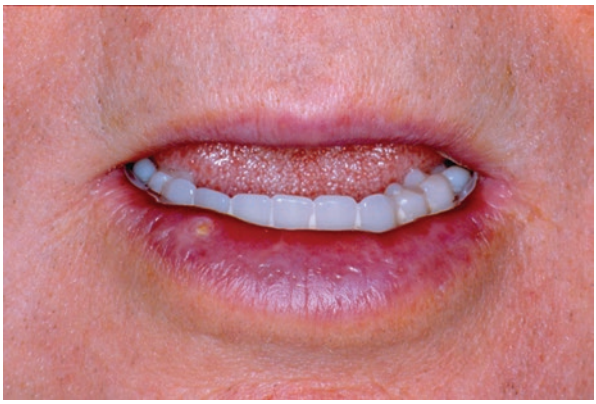

Fig. 9.9 Actinic cheilitis of the lower labial mucosa (@ John Wiley & Sons, reproduced with permission)

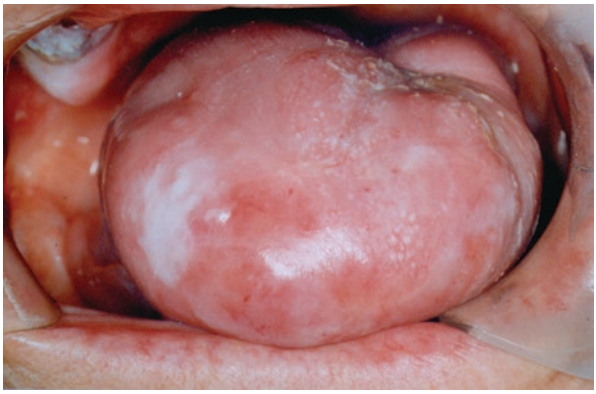

Fig. 9.10 Mucosal atrophy in tertiary syphilis (@ John Wiley & Sons, reproduced with permission)

Bone marrow transplant patients with graft-versus-host disease are also a vulnerable group as far as leukoplakia and erythroplakia are concerned. These patients are at high risk of malignant transformation.

Lichenoid Lesions and Lichen Planus

There are varying reports of the malignant potential of lichenoid lesions. Lichen planus is a multifactorial disorder which typically presents bilaterally with hyperkeratotic lesions comprising striae, nodules and plaques. Below the keratinised, atrophic superficial epithelial layers are acanthosis and a T cell infiltrate. If lesions undergo severe atrophy and basal cell liquefaction, red lesions containing erosions or bullae may appear. Lichenoid lesions can occur almost anywhere on the oral mucosa, but the most common sites are the buccal mucosa, the gingivae or the floor of the mouth and ventral tongue. The overall risk of malignant transformation for lichen planus is thought to be around 1%, with higher rates in atrophic or erosive

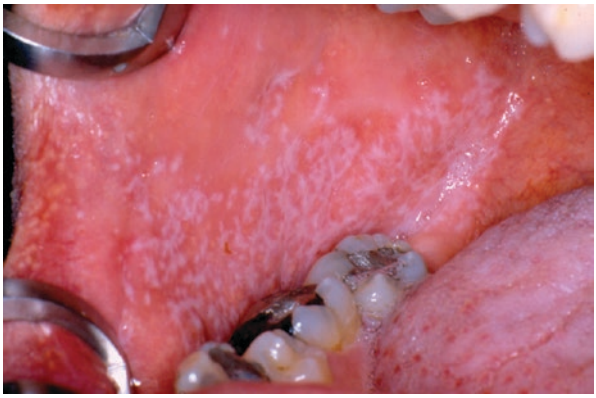

Fig. 9.11 A lichenoid lesion of the right buccal mucosa (@ John Wiley & Sons, reproduced with permission)

lichen planus where there is not a layer of protective surface keratinisation. It is not uncommon to see multiple oral premalignant lesion disease in patients with lichen planus or lichenoid lesions because extensive mucosal disease may predispose to a high risk of "field cancerisation".

Lichenoid lesions (Fig. 9.11) are clinically indistinguishable from lichen planus but can arise as hypersensitivity reactions to amalgam restorations or drugs. Unlike lichen planus, they do not tend to occur with bilateral presentation.

Given that lichenoid lesions and lichen planus can affect multiple sites of the oral mucosa, it is generally advised that all sites containing such lesions should be biopsied to rule out early malignant transformation or dysplastic changes which may give certain sites a predisposition to cancer development. It is not known why some lichenoid or lichen planus lesions undergo malignant transformation and others do not, but there is a tendency for dysplastic lesions especially those with an inflammatory cell infiltrate in the adjacent subepithelial tissue to be at high risk.

Goodson and Thomson reported in [23] a cohort of 88 patients with lichenoid inflammation who underwent excisional laser surgery. Of these, 60 displayed lesions with varying grades of dysplasia; despite interventional laser surgery, they, as a group, were significantly less likely to be disease-free after the follow-up period than other forms of OPMDs, and consequently this group may be prone to worse clinical outcome and poorer long-term prognosis.

Oral Submucous Fibrosis (OSMF)

This condition is commonly seen in South East Asia and related to betel quid use. Betel quid comprises areca nut mixed with slaked lime mixed in a betel vine leaf which is held in the mouth and acts as a stimulant. It is primarily used by manual labourers who may consume up to 30 quids a day but is also unfortunately consumed by children. The most obvious effects of betel quid are tooth discolouration and redness of the oral mucosa which may also exist in conjunction with

erythroplakia or leukoplakia. Over time, the oral mucosa becomes pale with less vascularity and hardened or fibrotic giving it a rubbery firm texture. The overlying epithelium becomes atrophic and has a high risk of malignant transformation of 0.5% [24]. Consequentially, OSMF and squamous cell carcinomas are commonly found at the site of betel quid placement in the sulcular epithelium and buccal mucosa.

Sideropenic Dysphagia

This uncommon condition commonly manifests in middle-aged women who have symptoms of dysphagia from oesophageal web formation, iron deficiency anaemia, glossitis and dysplastic lesions of the oral mucosa.

Discoid Lupus Erythematosus

Discoid lupus erythematosus is a chronic autoimmune condition predominantly affecting females with a characteristic red facial "butterfly rash" across the nose and cheeks. The classical premalignant oral lesion in DLE is a stellate lesion of the buccal mucosa, but patients may also present with circular areas of redness or ulceration of the oral mucosa, and these lesions characteristically have white borders so may be confused with lichen planus or erythroplakia.

Actinic Cheilitis

These lesions are usually crusted ulcerated lesions covering the lower lip. They are commonly found in people who have spent a lot of time outdoors in prolonged periods of exposure to UV and sunlight. The malignant transformation rates for actinic cheilitis are unclear, but most studies suggest squamous cell carcinoma develops from dysplastic tissue. There is some evidence that the absence of cytokeratin 10 predisposes to malignant transformation [25].

Clinically, actinic cheilitis can be confused with lichen planus and lip leukoplakia due to immune compromise.

Chronic Hyperplastic Candidosis (Candida Leukoplakia)

This condition commonly presents bilaterally with nonhomogeneous leukoplakia or erythroplakia at the labial commissures in smokers. Candida hyphae invade parakeratinised mucosa and can give rise to cellular atypia or varying degrees of dysplasia.

It is not clear whether dysplastic tissue provides a foundation for candida growth or whether the reverse is true, and tobacco and candida carcinogens create tissue dysmaturation and disorganisation (dysplasia).

Syphilis

This is an uncommon condition in the west but more common in Asian countries. The clinical presentation of tertiary syphilis may include leukoplakia of the dorsum of the tongue with a very high risk of malignant transformation.

Dyskeratosis Congenita

This rare condition of likely recessive inheritance affects males. The presentation may include greyish-brown skin pigmentation, immune deficiency, nail dystrophy and oral leukoplakia. The commonly affected sites in children are the tongue and buccal mucosa with vesicles or ulcerations. In later life, there is reddening of the mucosa and then erosive leukoplakia with high malignant potential in men aged 20–30.

Multiple Lesion Disease (Fig. 9.12)

A particularly difficult group of potentially malignant disorder patients to manage are those with multiple precancerous red and/or white lesions often comprising tissue that exhibits dysplastic change. Multifocal disease was first described by Slaughter [26] who popularised the idea that some patients have molecularly altered preneoplastic fields of the oral mucosa from which multiple lesions can develop either synchronously or metachronously. Multiple lesion disease has been reported to affect between 3% and 24% of patients with oral precancerous lesions and demonstrates malignant transformation rates of more than 20% [27–29].

In the study by Hamadah et al. [27] undertaken in the northeast of England, 78 patients with single and 18 with multiple lesions were assessed to see how many developed oral squamous cell carcinoma. Oral squamous cell carcinoma developed in 3/78 single lesions and 4/18 multiple lesions. Single lesions were most common on the floor of the mouth and ventrolateral tongue, and multiple lesions were more common on the buccal mucosa. Interestingly, the most severe dysplasia was found in single lesions, and these lesions had higher cyclin-A and Ki-67 labelling indices than the multiple lesions, yet a smaller proportion developed cancer over the 5-year follow-up period (3.8% of single lesions versus 22.2% of multiple lesions).

In contrast to single potentially malignant lesions which predominantly affect the floor of the mouth and ventral tongue, multiple lesion disease is more common

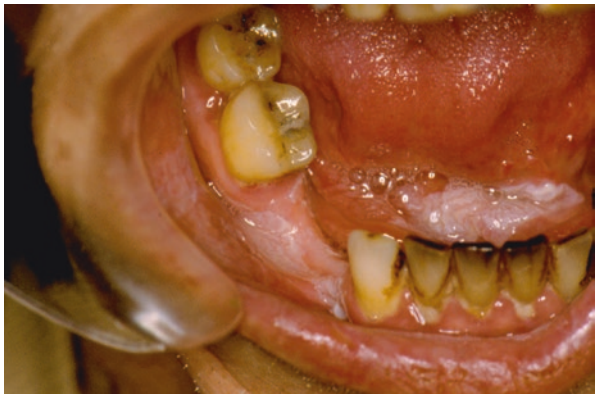

Fig. 9.12 Multiple lesion disease affecting the floor of the mouth and mandibular alveolus (@ John Wiley & Sons, reproduced with permission)

on the buccal mucosa, soft palate retromolar area and dorsum of the tongue. While precancerous conditions associated with systemic illness may be responsible for some cases of multiple lesion disease, there is also evidence that diet (high intake of fruit and vegetables) can have a protective effect on the development of multiple lesion disease [30, 31].

High- and Low-Risk Lesions

Risk Profiling of Oral Potentially Malignant Lesions

Assessment of malignant transformation risk for potentially malignant disorders is crucial and fundamental to the management of these conditions. Unfortunately, in clinical practice, the risk of malignant transformation remains obscure and highly variable, with quoted rates ranging from 0.13% to 36.4%. Mehanna et al. [32] reported an overall malignant transformation rate of around 12%, rising to 14.6% in patients whose lesions were left in situ, versus only 5.4% when the lesions were surgically excised. Thomson et al. reported transformation rates of between 2% and 4% in laser-treated patient cohorts, supporting the hypothesis that appropriate intervention helps to reduce the risk of cancer development.

In [33], Goodson et al. reported findings from a retrospective study of 1248 patients with oral cancer identified over a 13-year period. Of these, 58 patients had identifiable precursor lesions that became malignant, but only 25 had been dysplastic on initial biopsy. Nineteen of the 33 non-dysplastic lesions exhibited lichenoid inflammation only. SCC arose most often on the ventrolateral tongue and floor of the mouth, with a mean transformation time of 29.2 months. Transformation time was significantly shorter in men ($p = 0.018$) and those over 70 years of age ($p = 0.010$). Patients who consumed more than 21 units of alcohol/week and those

who had had interventional laser surgery to treat precursor lesions had higher-staged tumours ($p = 0.048$). This study showed that the results of incisional biopsy and grading of dysplasia had limited use as predictive tools and supported the view that cancer may arise in the absence of recognisable epithelial dysplasia. Consequently, risk profiling individual patients is difficult due to the lack of objective definitive clinical or pathological markers for malignant transformation and the unknown additive interactions in patients with multiple known clinicopathological risk factor variables.

There are currently no definitive studies quantifying risk for individual patients. Ideally, risk could be evaluated by a scoring system where patients were assessed for risk using weighted variables. The best evidence for risk factors comes from publications summarising findings of cohort and case-control studies where patients have been prospectively followed or retrospectively analysed to look at the effects of known or proposed risk factors on malignant transformation. It has however been difficult to examine the weighted effects of individual variables because many patients possess a number of risk factors that make them susceptible to malignancy. The combined effects of multiple risk factors can be difficult to quantify because they may not be additive but multiplicative and there are insufficient numbers of studies to accurately quantify these combined effects.

When assessing patients at presentation for high- or low-risk status, the assessment is based on the clinical history and examination findings as well as pathological examination of a biopsy specimen. As a consequence, overall risk profiling requires the clinician to take into account both clinical and histopathological factors.

Clinical Risk Profiling

In 2007, van der Waal identified factors that would suggest a patient is at statistically significant higher risk of malignant transformation of potentially malignant disorders (Table 9.2).

Almost 10 years later, these and other risk factors were further stratified into high- and low-risk categories. Diajil and Thomson [34] undertook a systematic review of 300 papers on oral cancer risk factors published between 1982 and 2009 and stratified 14 different risk factors as high or low risk. The higher-risk factors were tobacco use, excess alcohol consumption, use of betel quid, predisposing genetic factors and inherent susceptibility, immunodeficiency, diet low in fresh fruit and vegetables, old age and marijuana use. Low-risk factors in an individual patient included low socioeconomic status, poor oral health, use of shammah/toombak, human papillomavirus infection, *Candida albicans* infection and diabetes mellitus (Table 9.3).

The INHANCE study [35], published in 2012, looked at diet and head and neck cancer risk. In this analysis, pooled data included 22 case-control studies with

Table 9.2 Statistically significant risk factors for malignant transformation of oral potentially malignant disorders

Female gender
Long duration of leukoplakia
Nonhomogeneous leukoplakia or erythroplakia
Peripheral verrucous leukoplakia
Lesion size >200 mm^2
Presence and severity of epithelial dysplasia and dyskaryosis
Presence of *Candida albicans*
History of previous head and neck carcinoma or previous recurrent potentially malignant lesions
Multiple lesion disease
Patients with immune compromise
Tobacco smoking
Alcohol consumption
Low socioeconomic status and educational attainment
Unemployment
Age >40 years

Table 9.3 High- and low-risk factor profiling

High risk	Low risk
Tobacco use	Low socioeconomic status
Excess alcohol consumption	Poor oral health
Use of betel quid	Use of shammah/toombak
Predisposing genetic factors and inherent susceptibility	Human papillomavirus infection
Immunodeficiency	*Candida albicans* infection
Diet low in fresh fruit and vegetables	Diabetes mellitus
Old age	
Marijuana use	

14,520 cases and 22,737 controls. Centre-specific quartiles among the controls were used for food groups, and frequencies per week were used for single food items. A dietary pattern score combining high fruit and vegetable intake and low red meat intake was created. The study found that higher dietary pattern scores, reflecting high fruit/vegetable and low red meat intake, were associated with reduced head and neck cancer risk.

Regarding employment and socioeconomic status in risk factor profiling, the ARCAGE study evaluated the association between occupational history and upper aerodigestive tract (UADT) cancer risk in the ARCAGE European case-control study [36]. The study included almost 2000 cases and controls with cancer of the oral cavity, oropharynx, hypopharynx, larynx or oesophagus. The study found that among men, there were increased risks for cancer in painters, bricklayers, workers employed in the erection of roofs and frames, reinforced concreters, dockers and

workers in road construction and cargo handling. Increased risks were also found for loggers and cattle and dairy farmers. Among women, there was no clear evidence of increased risks of upper aerodigestive tract cancer in association with occupations or industrial activities.

Pathological Risk Profiling

The risk of malignant transformation for an individual patient however also may depend on pathological features of a biopsied lesion. The gold standard for assessment of oral potentially malignant lesions is microscopic examination of haematoxylin- and eosin-stained sections for architectural and cytological features of epithelial dysplasia, but only 50% leukoplakias actually demonstrate features of dysplasia, and malignant transformation rates of dysplastic mucosa can range from 0% to 50%.

The diagnostic gold standard for oral potentially malignant disorders is the WHO classification. The 2005 classification identifies cytological and histological features of dysplasia shown in Table 9.4, but there is little evidence to suggest which architectural and cytological features should be weighted more highly to identify high- and low-risk OPMDs. There is also no category of provision within these features for the diagnosis of proliferative verrucous leukoplakia which is often multifocal and has very high risk of malignant transformation. Dysplasia grading remains subjective and is prone to inter- and intraobserver variability.

Historically, pathologists took into account a combination of microscopic features from those listed above and arrived at a grade; the worst site of a biopsy was scored although sampling errors may have affected reporting. Before grading of dysplasia became more standardised, lesions were described as mild, moderate or

Table 9.4 Cytological and architectural features of dysplasia (Adapted from [37])

Cytological features of dysplasia	Architectural features of dysplasia
Abnormal variation in nuclear size and shape (anisonucleosis and pleomorphism)	Loss of polarity
Abnormal variation in cell size and shape	Disordered maturation from basal to squamous cells
Enlarged nuclei and cells	Includes top to bottom change of carcinoma in situ
Hyperchromatic nuclei	Increased cellular density
Increased mitotic figures	Basal cell hyperplasia
Abnormal mitotic figures (abnormal in shape or location)	Dyskeratosis (premature keratinisation and keratin pearls deep in epithelium)
Increased number and size of nucleoli	Bulbous drop-shaped rete pegs
	Secondary extensions (nodules) on rete pegs

severe dysplasia relatively subjectively. Pathologists in different units would make their own grading systems and sometimes only diagnosed dysplasia when only two of the listed features were observed [38]. Others scored lesions depending on how many dysplastic features a biopsy expressed providing weighted scores for 13 microscopic features of dysplasia, namely:

1. Loss of polarity of basal cells
2. Presence of more than one layer having basaloid appearance
3. Drop-shaped rete ridges
4. Increased nuclear cytoplasmic ratio
5. Nuclear hyperchromatism
6. Enlarged nucleoli
7. Increased number of mitotic figures
8. Mitotic figures in abnormal form
9. The presence of mitotic figures in the superficial half of the epithelium
10. Cellular and nuclear pleomorphism
11. Irregular epithelial stratification
12. Loss of intercellular adherence
13. Keratinisation of single cells or cell groups in the prickle cell layer

Smith and Pindborg [39] weighted these features in lesions, and a maximum score of 75 for any one lesion could be obtained. They considered mild dysplasia to include scores of 11–25, moderate dysplasia from 26 to 45 and severe dysplasia in excess of 45, but the appropriate weighting given to each of the features was largely guesswork on what the authors felt was more or less indicative of severity. Some of the features were not specific for dysplasia and could be found in other conditions such as inflammation.

To reduce ambiguity in reporting, the WHO further subdivided categories of mild, moderate and severe dysplasia and carcinoma in situ with features they felt were appropriate for each category, but not all features had to be present for a lesion to be given a particular grade.

There is some controversy on whether carcinoma in situ is actually a premalignant condition as many believe it to be actual malignant change but without invasion. Microinvasive carcinoma is also difficult to diagnose as it is difficult to visualise in the early stages.

In addition to difficulties assigning weightings to various cytological and histological features of dysplasia, there is a considerable amount of evidence suggesting that dysplasia grading is subjective and prone to interobserver variability [40–42]. In one study by Karabulut et al. [41], interobserver agreement rates were in the range of 49–69% between four pathologists with kappa values showing poor to moderate agreement between pathologists. Diagnostic difficulty is particularly associated with grading of moderate dysplasia where features are not unilaterally mildly dysplastic or severely dysplastic. Malignant transformation rates for mild dysplasia may be less than 5% but for moderate dysplasia are 3–15% and for severe dysplasia around 16% with variability of 7–50% [43]. Decisions on management of the entire precancerous lesions is

often based on grading of dysplasia from incisional biopsy which has also been reported as fairly inaccurate with reports of only 56% agreement in diagnosis between incisional and definitive biopsies from 200 patients with single premalignant lesions. Discrepancies in diagnosis in 42 patients with multiple lesions totalled 11.9%. Holmstrop et al. [44] undertook a similar study and found that incision biopsy reports gave different degrees of dysplasia compared to the whole lesions with variability of around 49% again confirming that biopsies may not be being taken from the most severely dysplastic area of a lesion visible with the naked eye.

For patients with multiple lesion disease, field mapping biopsies are advocated [45]. Multifocal disease may ultimately affect up to 24% of oral cancer patients, and for holistic patient management, all sites where there are visible epithelial abnormalities should be biopsied, if necessary under general anaesthesia.

Diagnostic accuracy is also problematic with verrucous hyperplasia and proliferative leukoplakia (PVL). These lesions have gross hyperkeratosis with a verrucous or papillomatous surface. The lesions are exophytic and spread laterally. Verrucous hyperplasia is more localised, but the recurrent multifocal and progressive type, PVL, occurs at an average age of diagnosis of 62 years, and women are more commonly affected than men. PVL usually affects multiple sites but most commonly the buccal mucosa in women and the tongue in men. Many cases are resistant to all forms of medical and surgical treatments including laser surgery.

While PVL lesions do not demonstrate many features of cytological atypia and only 50% show evidence of dysplasia, 70% of lesions may progress to squamous cell carcinoma [14, 46]. Clinical history, multifocality and extent of the lesion are all important factors in diagnosis. The exophytic nature and lack of pushing invasive front distinguish it from verrucous carcinoma [37]. Another area of diagnostic difficulty, also reported by Speight [37], includes pseudoepitheliomatous lesions. Granular cell tumours are typical examples along with chronic hyperplastic candidosis, median rhomboid glossitis and necrotising sialometaplasia. Reactive inflammatory cell atypia is common in these lesions and should be differentiated from atypia in oral dysplasia.

In an attempt to standardise dysplasia classification and use it to predict risk of malignant transformation, Kujan et al. [47] developed a novel binary grading system where lesions were reclassified as low or high risk for malignant transformation. There was some success in using this classification in that it reduced the number of categories for a lesion down to two. Using the binary system, four pathologists showed satisfactory agreement on the distinction of mild dysplasia from severe dysplasia and from carcinoma in situ, but the assessment of moderate dysplasia was more difficult. The sensitivity and specificity of the new binary grading system for predicting malignant transformation in oral epithelial dysplasia were 85% and 80%, respectively, and the accuracy was reported as 82%. It was felt that the new binary grading system complemented the WHO Classification 2005 but needed further evaluation on a larger sample size.

In summary, there is no single dysplasia classification system at present that is more or less accurate than the WHO system. All systems use similar features to classify dysplasia, but there needs to be consensus agreement on which features are more indicative of more severe tissue dysmaturation and disorganisation. This combined with introduction of clinical factors to stratify risk may provide a more encompassing system that provides prognostic as well as diagnostic information.

A considerable amount of work has been undertaken trying to find biomarkers that predict malignant transformation at the molecular level, but to date, no accurate biomarkers have consistently been able to predict malignant transformation of oral potentially malignant disorders, and these are largely a research tool. They are not in routine use for individual patient management.

References

1. Pindborg JJ. Oral cancer and precancer. Bristol: John Wright and Sons; 1980.
2. Speight P, Farthing PM, Bouquot JE. The pathology of oral cancer and precancer. Curr Diagn Pathol. 1996;3:165–76.
3. Thomson PJ. Field change and oral cancer: new evidence for widespread carcinogenesis? Int J Oral Maxillofac Surg. 2002;31(3):262–6.
4. Thomson PJ. Oral Precancer. Diagnosis and management of potentially malignant disorders. Chichester: Wiley Blackwell; 2012.
5. Gupta PC, Herbert JR, Bhonsle R, Murthy PR. Influence of dietary factors on oral precancerous lesions in a population based case control study in Kerala, India. Cancer. 1999;85:21887–93.
6. Hashibe M, Sankaranarayanan R, Thomas G, Kuruvilla B, Mathew B, Somanathan T, Parkin DM, Zhang ZF. Alcohol drinking, body mass index and the risk of oral leukoplakia in an in Indian population. Int J Cancer. 2000;88(1):129–34.
7. Franco EL, Kowalski LP, Oliviera BV. Risk factors for oral cancer in Brazil: a case control study. Int J Cancer. 1989;43:882–1000.
8. Marshall JR, Graham S, Haughey BP, Shedd D. Smoking, alcohol, dentition and diet in the epidemiology of oral cancer. Oral Oncol Eur J Cancer. 1992;28B:9–15.
9. Scully C. Oral precancer: preventive and medical approaches to management. Oral Oncol Eur J Cancer. 1995;31B:16–26.
10. Zheng T, Boyle P, et al. Tobacco smoking, alcohol consumption and risk of oral cancer: a case control study in Beijing, Peoples republic of China. Cancer Causes Control. 1990;3:173–9.
11. Goodson ML, Thomson PJ. Tissue autofluorescence and the diagnosis and management of oral potentially malignant disorders. J Oral Maxillofac Surg Photon. 2014;117:205–14.
12. Bagan JV, Jiminez Y, Sanchis JM. Proliferative verrucous leukoplakia: high incidence of gingival squamous carcinoma. J Oral Pathol Med. 2003;32:379–82.
13. Silverman S, Gorky M. Proliferative verrucous leukoplakia: a follow up of 54 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1997;84:154–7.
14. Zakrzewska JM, Lopes V, Speight P. Proliferative verrucous leukoplakia: a report of 10 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1996;82:396–401.
15. Bagan JV, Jiminez Y, Diaz-Fernandez JM, Murillo-Cortes J, Sanchis Bielsa JM. Malignant transformation of proliferative verrucous leukoplakia to oral squamous carcinoma: a series of 55 cases. Oral Oncol. 2011;47(8):732–5.
16. Goodson ML, Thomson PJ. Management of oral carcinoma: benefits of early precancerous intervention. Br J Oral Maxillofac Surg. 2011;49:88–91.
17. Dietrich T, Reichart PA, Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. Oral Oncol. 2004;40(2):158–63.

18. Chen PC, Pan CC, Kuo C, Lin CP. Risk of oral nonmalignant lesions associated with human papillomavirus infection, betel quid chewing and cigarette smoking in Taiwan: an integrated molecular and epidemiologic study. Arch Pathol Lab Med. 2006;130(1):57–61.
19. Anantharaman D, Gheit T, Waterboer T, Abedi-Ardekani B, Carreira C, McKay-Chopin S, Gaborieau V, Marron M, Lagiou P, Ahrens W, Holcátová I, Merletti F, Kjaerheim K, Talamini R, Simonato L, Castellsague X, Macfarlane TV, Biggs AM, Thakker N, Znaor A, Thomson P, Canova C, Conway DI, Healy CM, Tommasino M, Pawlita M, Brennan P. Human papillomavirus infections and upper aero-digestive tract cancers: the ARCAGE study. J Natl Cancer Inst. 2013;105:536–45.
20. Diaz-Guzman L, Castellanos JL. Lesiones de la mucosa buccal. Estudio Epidemiologicao en 7297 pacientes. Rev ADM. 1991;48:75–80.
21. Prout MN, Sidari JN, Witzburg RA, et al. Head and neck cancer screening among 4,611 tobacco users older than 40 years. Otolaryngol Head Neck Surg. 1997;116:201–8. https://doi.org/10.1016/S0194-5998(97)70326-7.
22. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007;36(10):575–80.
23. Goodson ML, Thomson PJ. Oral lichenoid lesions: a significant diagnosis in oral potentially malignant disorder management? Int J Oral Maxillofac Surg. 2017;46(Suppl 1):305.
24. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45(4-5):317–23.
25. Garcia NG, Oliveira DT, Lauris JRP, Domingues MAC, Minicucci EM, Soares CT. Loss of cytokeratin 10 indicates malignant transformation in actinic cheilitis. Clin Oral Investig. 2015;20(4):745–52.
26. Slaughter DP, Southwick HW, Smejkal W. "Field cancerization" in oral stratified squamous epithelium. Clinical implications of multicentric origin. Cancer. 1953;6(5):963–8.
27. Hamadah O, Goodson ML, Thomson PJ. Clinicopathological behaviour of multiple oral dysplastic lesions compared with that of single lesions. Br J Oral Maxillofac Surg. 2010;48(7):503–6.
28. Lee JJ, Hong WK, Hittlelam WN. Predicting cancer development in oral leukoplakia: ten years of translational research. Clin Cancer Res. 2000;6:1702–10.
29. Saito T, Sugiura C, Hirai A, et al. High Malignant transformation rate of widespread multiple oral leukoplakias. Oral Dis. 1999;5:15–9.
30. Goodson ML, Hamadah O, Thomson PJ. The role of alcohol in oral precancer: observations from a North East England Population. Br J Oral Maxillofac Surg. 2009;47:507–10.
31. Thomas G, Hashibe M, Jacob BJ, Ramadas K, Mathew B, Sankaranarayanan R, Zhan ZF. Risk factors for multiple oral premalignant lesions. Int J Cancer. 2003;107:285–91.
32. Mehanna HM, Rattay T, Smith J, McConkey CC. Treatment and follow up of dysplasia- a systematic review and meta-analysis. Head Neck. 2009;31:1600–9.
33. Goodson ML, Sloan PS, Robinson CM, Cocks K, Thomson PJ. Oral precursor lesions and malignant transformation - who, where, what and when? Br J Oral Maxillofac Surg. 2015;53:831–5.
34. Diajil AR, Thomson PJ. Risk factors of oral cancer and potentially malignant disorders (PMDs) Developing a High/Low Risk profiling system. J Bagh College Dent. 2016;28(1):63–9.
35. Chuang SC, Jenab M, Heck JE, Bosetti C, Talamini R, Matsuo K, Castellsague X, Franceschi S, Herrero R, Winn DM, La Vecchia C, Morgenstern H, Zhang ZF, Levi F, Dal Maso L, Kelsey K, McClean MD, Vaughan T, Lazarus P, Muscat J, Ramroth H, Chen C, Schwartz SM, Eluf-Neto J, Hayes RB, Purdue M, Boccia S, Cadoni G, Zaridze D, Koifman S, Curado MP, Ahrens W, Benhamou S, Matos E, Lagiou P, Szeszenia-Dabrowska N, Olshan AF, Fernandez L, Menezes A, Agudo A, Daudt AW, Merletti F, Macfarlane GJ, Kjaerheim K, Mates D, Holcatova I, Schantz S, Yu GP, Simonato L, Brenner H, Mueller H, Conway DI, Thomson P, Fabianova E, Znaor A, Rudnai P, Healy CM, Ferro G, Brennan P, Boffetta P, Hashibe M. Diet and the risk of head and neck cancer: a pooled analysis in the INHANCE consortium. Cancer Causes Control. 2012;23:69–88.

36. Lagiou P, Talamini R, Samoli E, Lagiou A, Ahrens W, Pohlabeln H, Benhamou S, Bouchardy C, Slamova A, Schejbalova M, Merletti F. Diet and upper-aerodigestive tract cancer in Europe: the ARCAGE study. Int J Cancer. 2009;124(11):2671–6.
37. Speight PM. Update on epithelial dysplasia and progression to cancer. Head Neck Pathol. 2007;1:61–6.
38. Banoczy J, Csiba A. Occurrence of epithelial dysplasia in oral leukoplakia. Oral Surg Oral Med Oral Pathol. 1976;42:766–74.
39. Smith C, Pindborg JJ. Histological grading of oral epithelial atypia by the use of photographic standards. Copenhagen: C. Hambergers Bogrykkeri; 1969.
40. Abbey LM, Kaugars GE, Gunsholly IC. Interexaminer and intra examiner reliability in the diagnosis of oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol. 1995;80:188–91.
41. Karabulut A, Reibel J, Therkildsen MH, Praetorius F, Nielson HW, Dabelsteen E. Observer variability in the histologic assessment of oral premalignant lesions. J Oral Pathol Med. 1995;24:198–200.
42. Pindborg JJ, Reibel J, Holmstrup P. Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. J Oral Pathol. 1985;14:698–708.
43. Scully C, Sudbø J, Speight PM. Progress in determining the malignant potential of oral lesions. J Oral Pathol Med. 2003;32(5):251–6.
44. Holmstrop P, Vedofte P, Reibel J, Stoltze K. Longterm treatment outcome of oral premalignant lesions. Oral Oncol. 2006;42:461–74.
45. Thomson PJ, Hamadah O. Cancerisation within the oral cavity: the use of ‘Field Mapping Biopsies’ in clinical management. Oral Oncol. 2007;43:20–6.
46. Cabay RJ, Morton TH, Epstein JB. Proliferative verrucous leukoplakia and its progression to oral carcinoma: a review of the literature. J Oral Pathol Med. 2007;36:255–61.
47. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006;42(10):987–93.

Chapter 10
Surgical Biopsy Techniques and Adjuncts

Ben Tudor-Green

Introduction

The significance of oral potentially malignant disorders (OPMD) lies in its association with malignant transformation to oral squamous cell carcinoma (OSCC) [1]. OPMD can present in a number of ways, as homogeneous (flat, thin, white) and non-homogeneous (speckled, red and white, erythroleukoplakia) lesions but can also present as oral submucous fibrosis and oral lichenoid/oral lichen planus (OLP). OPMD is diagnosed by clinical history and biopsy with histological examination. Prior to biopsy, it is important to be aware if a patient is on anticoagulant or antiplatelet therapy as these may have to be stopped for a number of days and should seek advice from a Haematologist if unsure [2]. There also is a need to be aware of any anatomical structures at risk. If situated at the tongue base or oropharynx, an examination under anaesthetic is performed, and if a lesion is situated proximal to major structures, a biopsy may be contraindicated. Biopsy remains the gold standard and is important in helping exclude other keratotic lesions. It can present as a spectrum of epithelial change. The most commonly used grading system is the WHO 2005 system that grades dysplasia into mild, moderate and severe [3]. The use of a binary system has been suggested for reducing interobserver variability of histological grading and for helping guide clinical decisions for appropriate intervention, and a later study confirmed its superior reproducibility [4, 5]. However, Dost et al. argued that the severity of OPMD was not associated with predicting patient outcomes or determining appropriate management and advised treatment [6]. Non-homogenous leukoplakias are associated with a higher risk of dysplasia or OSCC compared to homogeneous lesions. Proliferative verrucous

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leukoplakia (PVL) is a subtype and has a higher degree of malignant transformation [1, 7]. It has been suggested that in the absence of dysplasia, it can be a precursor to verrucous carcinoma [8].

The global prevalence of oral leukoplakia is estimated at 2.60% [9]. Most OPMDs such as leukoplakia are caused by tobacco, alcohol and betel quid nut use resulting in the sequential revolution of the disorder. The anatomical subsite can be considered a factor for treatment. Warnakulasuriya and Ariyawardana identified that the floor of the mouth lesions carry a greater risk of malignant transformation [9].

The significance of OPMD lies in the risk of malignant transformation into OSCC. It has been reported in the literature as occurring from 6.6% to 36.4% [1] alone to 0.13–34.0% [9]. The average time to transformation has been reported in the literature varying from 0.5 to 17 years [10, 11]. Mild OPMD is associated with a less than 5% malignant transformation compared to moderate and severe OPMD where there have been rates reported of 3–15% and 7–50%, respectively [12, 13]. The risk factors associated with increased malignant transformation include female gender, duration of lesion, idiopathic leukoplakia, subsite (floor of the mouth), size (greater than 200 mm) and multiple lesions. Ho et al. reported that 22% of the patients underwent malignant transformation within 5 years. However, gender, age and number of lesions did not predict malignant transformation [10].

There is a general lack of consensus as to the management of OPMD lesions [14–16], and very few guidelines have been published [1]. Surgical excision is often carried out for high-grade lesions and has been reported in the literature that if lesions are not excised, then there is an increased risk of malignant transformation [17], but there is controversy as to low-grade and high-grade lesions [18]. In the UK, the general advice given by the British Association of Head and Neck Oncologists (BAHNO) is for targeted use of biopsy and histological assessment followed by lifestyle changes (smoking and alcohol cessation) and surgical excision. A recent editorial produced an algorithm (Fig. 10.1) defining their management of OPMD [1] which has produced good results [11]. The aim of this chapter is to assess the biopsy techniques (Table 10.1) and adjuncts available to the clinician in the assessment of OPMD.

Fine Needle Aspiration Cytology (FNAC)

FNAC can be performed either blind or under imaging guidance. It is minimally invasive, fast inexpensive and well tolerated. The diagnostic performance and specificity of FNAC can be improved significantly when used in conjunction with a cytology technician-led service or with a cytologist where the sample can be processed and assessed immediately and lesions can be re-aspirated if required. Ultrasound-guided FNAC increases diagnostic accuracy by enabling avoidance of necrotic or cystic regions and the targeting of high-yield areas of the lesion for tissue extraction [19]. Ultrasound guidance can also allow the operator to confirm the position of the needle tip in the lesion. The major disadvantage is that FNAC

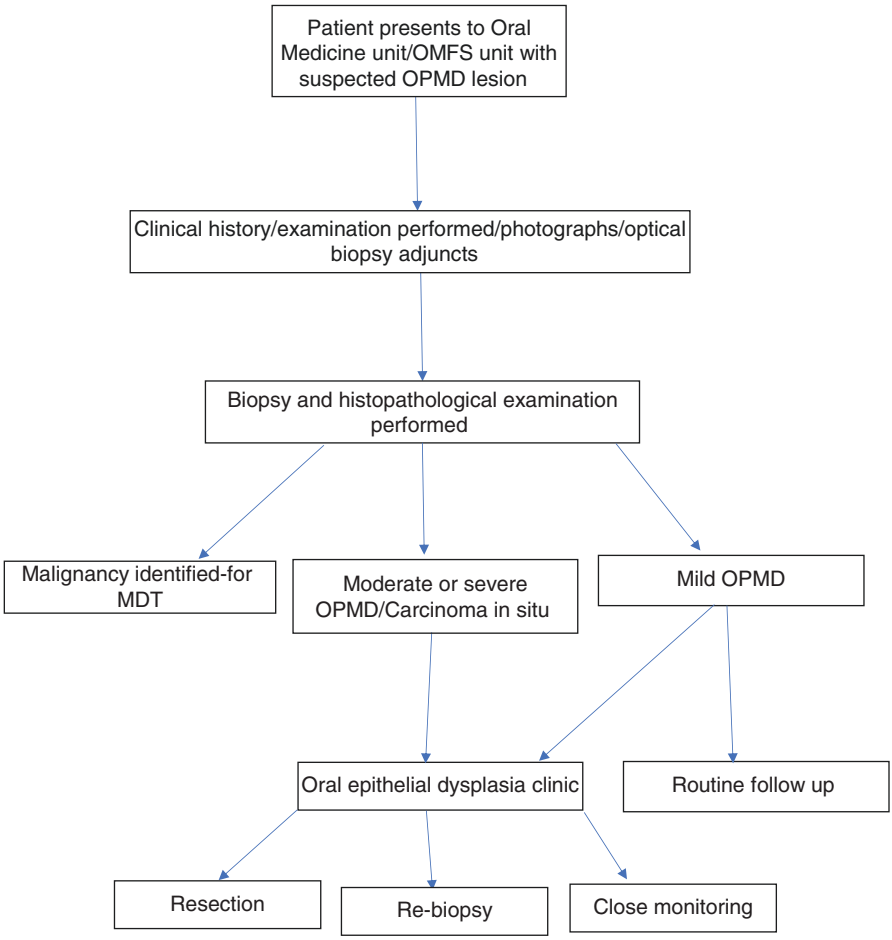

Fig. 10.1 Typical management algorithm for OPMD (based on Field et al. 2015) [1]

provides variously clustered cells and no tissue architecture and supply stroma, and it is the architectural presentation in a biopsy sample that allows for accurate diagnosis. One of the issues with FNAC is that it can induce changes within tissue causing diagnostic difficulties during final histological assessment. This applies in particular to glandular tissue for salivary gland assessment [20].

There are limited studies exploring this approach, but case reports [21] have shown that there was a correlation between the cytological findings and histological results achieving sensitivity of 100% and specificity of 89% which is comparable to earlier studies such as Gandhi et al. where the specificity and sensitivity were 95.45% and 93.75%, respectively [22]. This approach may be useful for lesions at the floor of the mouth or base of the tongue as it is less invasive and can be diagnostic in the majority of cases [23].

Table 10.1 Advantages and disadvantages of surgical biopsy techniques

Biopsy technique	Advantages	Disadvantages
Brush biopsy	Non-invasive Cheap No anaesthesia required	Non-definitive results Need histopathological diagnosis
Microbiopsy	Non-invasive Can be used in the primary care setting No anaesthesia required	Needs definitive histopathological diagnosis
Fine needle aspiration cytology (FNAC)	Minimally invasive Can be used in the outpatient setting Suitable for use for patients on anticoagulants/antiplatelets	May need definitive histopathological diagnosis May need imaging guidance
Core biopsy	Can be used in the outpatient setting Suitable for use for patients on antiplatelet/anticoagulants Suitable for floor of mouth/tongue base OPMD	Invasive May need imaging guidance
Incisional biopsy	Most common performed technique Can be performed in day case setting	Invasive Risk of underdiagnosis May need excisional biopsy Needs local or general anaesthesia
Excisional biopsy	Achieve potential curative intent Able to assess full extent of lesion Can be performed in day case setting	Invasive Needs local or general anaesthesia

Brush Biopsy

Oral brush biopsy may be used as an adjunct to incisional biopsy for lesions where there is uncertainty in the aetiology, continuing monitoring, recurrence or difficultly to excise surgically [24]. It can also be used in resource-challenged areas [25]. Various tools can be used from a Cytobrush to a toothbrush to collect cells. The removed cells are then transferred onto a glass slide and examined [26]. Trakroo et al. assessed 50 patients who had brush biopsy followed by incisional biopsy. The authors achieved a specificity of 88.89% and a sensitivity of 84.37%. In comparison with histopathology, there was a statistically significant difference ($P < 0.05$) [26]. This compares with earlier studies such as where the specificity and sensitivity were similar but no statistically significant difference between histopathology and cytology but overall has higher efficacy than cytology [25, 27]. In a recent meta-analysis comparing computer-assisted brush biopsy with DNA cytometry, the pooled sensitivity (86% vs. 89%) and specificity (81% vs. 99%) of brush biopsy were lower. The authors commented on the greater accuracy of DNA cytometry in its ability to detect DNA aneuploidy and detect malignant transformation sooner [28].

Microbiopsy

This can be performed either in the general dental setting or in the outpatient clinic and consists of scraping the lesion with a dermatological curette to collect cells and tissue. The basal layers of the epithelium have to be included for a full thickness tissue sample, so bleeding is encouraged [29]. The specimen is then placed in a fixing solution [26]. The authors comment that it should replace incisional biopsy but should be available in order to reduce diagnostic delay. The major disadvantage is the inadequacy of the depth of sampling resulting from keratinisation. The study had an inadequacy rate of 7.9% which is comparable to an earlier study [29, 30].

Core Biopsy

Owing to the diagnostic limitations with FNAC has led to exploration of core biopsy. The technique requires an operator which is usually a radiologist trained in head and neck ultrasound and biopsy techniques [19, 31]. Core biopsy consists of local anaesthetic infiltration, a small skin incision and the use of an automated spring-loaded biopsy device. The core of tissue is then processed and sections from the core can be used for grading [19]. The advantages of core biopsy are that it is rapid, the affected lesion remains in situ and no general anaesthetic is required. Also, it is suitable for patients on anticoagulation or antiplatelet therapy. The problems however are false-positive results, the minimal risk of tumour seeding and complications associated with a more invasive technique. A recent meta-analysis [32] has identified the crude estimates for tumour seeding of 0.00012% after FNA and 0.0011% after core needle biopsy (CNB).

CNB cannot be used as part of a one-stop diagnostic clinic service unlike FNAC. However, Howlett et al. argue that it should be the first diagnostic technique of choice compared to FNAC particularly for salivary gland lesions and cervical lymphadenopathy [33, 34], but despite limited data with this approach in OPMD, it has been shown in case reports to be useful where there is a suspicion that the tumour is entering a deeper tissue plane but the mucous membrane remains intact. It can be used to help evaluate the dimensions of a lesion especially in the tongue and can be used for assessing recurrent lesions [35]. Bleeding and pain are potential complications. However the risk can be minimised by ultrasound guidance. One study assessed the complications and diagnostic accuracy of ultrasound-guided core biopsy in patients who received antiplatelet therapy against those who did not. Thirty-two patients were either on aspirin, clopidogrel or warfarin. On follow-up none of the patients developed bleeding or haematoma in the antiplatelet/anticoagulant group. Despite the small cohort of patients, core biopsy could be used, but further studies are needed [36].

Novoa et al. performed a systematic review and meta-analysis of data from 16 studies involving 1291 lesions in 1267 patients. Overall, there was an accuracy of 94%, and it was able to provide a comment-specific diagnosis in 87% of cases without major complications and was seen as more accurate than FNAC in detecting malignancy. However, the authors concluded that CNB is suitable primarily for assessing salivary gland lesions and cervical lymphadenopathy but not suitable for thyroid lesions [36]. A recent study performed a retrospective analysis of 70 patients with neck masses over a 17-month period. The authors showed that 63 (90%) were diagnostic for lymphoma or other pathology and did not require further tissue sampling. The conclusion was that CNB allowed for assessment of full nodal architecture and advised that it should be the first investigation of choice as it reduces the need for surgical biopsy [37].

Incisional Biopsy

Incisional biopsy is usually indicated for determining the diagnosis prior to treatment for lesions that are potentially malignant or for lesions where the diagnosis is uncertain. The contemporary management of OPMD is incisional biopsy prior to surgical excision [14] although there is no universal consensus. Incisional biopsy has been shown to be of limited use as a predictive tool for oral dysplasia [38, 39]. There is also the danger of underdiagnosis, and it has been shown that the clinical appearance that appears non-homogeneous can influence underdiagnosis [40–42]. This was confirmed by Jeong et al. in the assessment of 22 patients with oral leukoplakia in the lateral tongue who found that 59.1% had co-existing malignancy and 73.3% of cases were underdiagnosed by incisional biopsy and identified three possible causes: first misselection of the sampling site within the area of leukoplakia, second, small sample size and third too superficial sample [43]. One way to reduce the risk of error is to place a tagging suture to facilitate the pathologist in orientation, facilitate deeper biopsy and reduce distortion [44]. Another issue is that by taking from the central aspect of a lesion, the report will give an approximate thickness as opposed to depth. If a lesion is malignant, depth is considered a better prognosticator than thickness [45].

Excisional Biopsy

This is useful for excising simple mucosal and soft tissue lesions where diagnosis and curative intent can be achieved simultaneously. However, this technique should only be used where the lesion can be removed without damage to vital structures. Excision of OPMD lesions takes place after initial incisional biopsy, and histopathological diagnosis as the severity of dysplasia varies at different sites across the lesion [1, 14, 41]. Mehanna et al. [17] suggested that excision should be advised for most lesions.

Chronic Hyperplastic Candidiasis (CHC)

CHC is a variant of oral candidiasis and classically presents as a white patch on the commissures of the oral mucosa [46, 47]. This is mainly caused by *Candida albicans*, but other candidal species can be involved. This is a normal commensal of the skin, oral cavity, gastrointestinal tract and genitourinary tract [47, 48]. CHC generally responds to antifungal therapy but if left untreated can lead to dysplasia and carcinoma. The association between *Candida* and dysplasia was first reported in the 1960s. Lehner first used the term "candidal leukoplakia" to describe leukoplakic lesions associated with candidal infection [48]. This term is no longer used. Males are generally more affected than females (2:1 ratio), and the buccal commissure is the most common site followed by the buccal mucosa and palate. The tongue is less likely to be affected [46].

Smoking is the main risk factor associated with CHC [49]. Shin et al. reported a positive and direct association between smoking and candidal colonisation. The accepted theory is that smoking allows candidal colonisation by epithelial alterations such as keratinisation and hydrophobicity [50]. An alternative theory is that smoking depresses the action of polymorphonuclear leucocytes resulting in reduced gingival exudate causing a reduced number of leucocytes and salivary immunoglobulin A levels which are needed for inhibiting candidal colonisation [51].

Both homogeneous and nodular types of CHC can result in moderate to severe dysplasia. It has been reported in the literature that up to 15% of CHC cases progress to dysplasia [46]. Field et al. in 1989 suggested that the nitrosamine that is produced by *Candida* with other carcinogens can activate proto-oncogenes [51]. A more recent study revealed that in patients with OPMD and *Candida*, there was more acetaldehyde production at carcinogenic levels [52]. McCollough et al. showed that there is an interaction between oral yeast carriage and oral epithelial dysplasia. The authors identified those patients with oral epithelial dysplasia or oral squamous cell carcinoma had an increased number of *Candida* in the oral cavity. They further commented on increased expression of p53 in CHC suggesting increased potential for malignant change in the epithelium [53].

Candidal colonisation in the oral cavity in itself is not indicative of infection as it is present in the locations described in between 40% and 60% of people [53]. Definitive diagnosis requires incisional biopsy to confirm tissue invasion. Biopsy is required as the lesion is often confused clinically with leukoplakia [54]. Histopathological examination of tissue reveals epithelial hyperplasia with hyperparakeratosis and candidal hyphae in the para-keratin layer invading the deeper layers of epithelium [38]. There can also be an inflammatory cell infiltrate consisting of polymorphonuclear leucocytes with lymphocytes in the lamina propria and the superficial layer of the epithelium. The presence of hyphae is confirmed with periodic acid-Schiff (PAS) staining which stains magenta red [46].

The management of CHC consists of eliminating predisposing factors such as smoking, reducing antibiotic therapy and controlling diabetes plus topical or systemic antifungal therapy. Surgery is used in cases that are nonresponsive to medical treatment [46, 47, 54].

Immunohistochemistry (IHC)

IHC examination has advantages in that it does not need specialist equipment and lengthy manipulation of tissue samples and can be applied to archived specimens and complements histopathological examination by detecting gene expression at the protein level [55]. Tumour suppressor genes, oncogenes, cell proliferation markers and cell adhesion molecules have been studied as potential tools to predict the prognosis of OPMD patients. An immunohistochemical panel using multiple prognostic molecular biomarkers could provide information for patient stratification [55]. The interpretation and quantification of IHC results are governed by various factors including examiner's experience, tissue processing, antibody specificity and antibody detection systems [55].

Ki67 is a commonly used marker associated with cell proliferation. A recent study [56] analysed the expression of Ki67 with survivin and p63 in oral leukoplakic tissues. The authors identified that there was a high expression of p63 (88.2%) in both dysplastic lesions and non-dysplastic lesions. There was a correlation between the high expression of survivin and Ki67 in dysplastic lesions which suggests that these markers could be used to assess malignant transformation. This is consistent with other studies where Ki67 was highly expressed in non-homogeneous lesions, but there was an increased indication in patients over 50 who smoked tobacco. It could be argued that Ki67 may be considered as an adjunct marker for assessing proliferative activity in lesions with malignant potential [57]. Ki67 can also be used for assessing malignant transformation in oral lichenoid and OLP in combination with p53 where there can be an increase in p53 and Ki67 expressions but not found to be statistically significant [58]. P53 in combination with p16 protein loss and Ki67 overexpression can increase the positive predictive value [59]. An alternative marker is topoisomerase II alpha which is more specific in identifying dysplasia as Ki67 can be expressed in 36% of the cells. However, topoisomerase II alpha was significantly associated with dysplasia ($P = 0.019$) in the samples [60].

Bcl-2 which is an oncoprotein can be overexpressed in potentially malignant oral lesions [61]. Its increased expression suggests that apoptosis may play a major role in the early stages of carcinomatosis [62]. Silva et al. assessed the correlation between increased expression cell adhesion markers (E-cadherin) and cell differentiation markers (involucin) in potentially malignant lesions. There was no statistically significant correlation between the expression of E-cadherin and involucin, but E-cadherin was highly expressed in leukoplakic lesions and involucin in oral SCC lesions [63]. Earlier studies confirm these findings in that they have shown significant differences in E-cadherin expression between normal oral mucosa and both low- ($P = 0.019$) and high-risk oral leukoplakia ($P = 0.006$). This can be seen with other oral lichen planus (OLP) and oral lichenoid lesions [64] where there was a higher expression in the OLP group which indicated increased malignant transformation, but generally affects a small subunit of patients predominately older and female.

LAMC2 has been shown to play a role in cancer invasion and is upregulated at the cancer/stroma interface. In a study of 39 surgical specimens and 93 incisional

biopsy specimens, LAMC2 expression was significantly associated with depth and invasion of oral SCC, and a foci of LAMC2-positive cells in some cases of leukoplakia were identified suggesting increased risk of malignancy [65]. Chaudhari et al. assessed the use of human MutL homolog 1 (hMLH1) protein expression in oral leukoplakia. This protein constitutes a part of maintaining genomic stability during repeated duplications. The authors identified an inverse relationship between hMLH1 expression and the degree of dysplasia. Overall, there is a lower expression in oral SCC compared to normal oral tissue. This could be used as part of a immunohistochemical panel for malignant transformation [66].

Molecular Markers

Recent advancements in genome-wide screening techniques have enabled identification of DNA aberrations associated with malignant transformation. Many studies have focused on oral leukoplakia and erythroplakia. Copy number variations (CNV) are abnormal duplications or deletions that exist across the genome [59]. One study identified a −8p/+3q/+8q/+20 phenotype in dysplastic lesions, suggesting that the pattern of genomic instability occurs at the premalignant stage [67]. A separate study identified further CNV including amplifications of 3p26.3, 8q24.1 and 11q22.3 and deletion of 8p23.2. Further amplifications of 1p36.33 and 11q22.1 are associated with poor clinical outcome [68].

Loss of heterozygosity (LOH) can occur upon loss of genomic material. LOH has been identified at chromosomal areas 3p, 9p, 13q and 17p in the early stages of oral carcinogenesis [69, 70]. Several studies have confirmed that high-frequency LOH particularly in 3p and 9p is positively correlated with malignant transformation. The risk is further increased if there is involvement of 8p, 11q, 13q and 17p. LOH has the potential to be used to improve surveillance of high-risk patients [71].

Several genes and proteins have been identified as potential markers for both dysplasia and malignant transformation. The most studied to date are p53, cyclin D1 and podoplanin (PDPN) [59]. The mutation of p53 is one of the most common genetic events in carcinogenesis. Around 50% of leukoplakia and erythroplakia overexpress p53. However, it cannot be used as a specific marker to determine malignant transformation and should be used with immunohistochemistry markers or DNA ploidy analysis [59]. Cyclin D1 has been shown to play a role in apoptosis and controls the cell cycle transition from G1 to S phase. Cyclin D1 gene overexpression has been found in 24–47% of dysplastic lesions [72], and Poh et al. showed an eightfold increase in malignant transformation with overexpression [73]. The correlation between cyclin D1 expression and dysplasia severity has been seen in several studies [74], but other studies have contraindicated these findings. The result is due to the differences in the grading scales used [59].

Podoplanin (PDPN) is described as a mucin-type transmembrane glycoprotein that may have a role in cell motility and platelet aggregation. In a study of

leukoplakia and erythroplakia lesions, there was positive correlation between the intensity of the staining and the severity of dysplasia [75] which has been confirmed in both more recent studies on non-homogeneous dysplasia [76] and earlier studies [77] where PDPN overexpression was associated with increased malignancy risk ($P = 0.0033$). A few studies have reported separately on erythroplakia [78] and leukoplakia [79]. PDPN and ALDH1 can be used in conjunction to identify increased malignant risk where they saw a 3.02-fold increase on point prevalence analysis of malignant transformation [80].

Epigenetics

An example of epigenetic changes in potentially oral malignant lesions is DNA methylation [81]. This occurs primarily in the addition of methyl group to a CpG dinucleotide in the DNA sequence. A recent meta-analysis examined DNA methylation in premalignant lesions and identified hyper-methylated loci on p16, p14, MGMT and DAPK leading to an increased risk of malignant transformation. The authors concluded that most studies were small in sample size and larger epigenome-wide studies are needed [82]. Earlier studies confirm that MGMT and p16 involvement increases risk [83]. Other recent studies identified that the methylation of genes including MPLP, NKX 2-3 and TRPC4 in their promoter genes leads to aberrant promoter methylation and therefore increased malignant transformation [84].

DNA Ploidy Analysis

DNA ploidy analysis can be used to measure nuclear DNA content as a marker of genomic damage [60]. It is known that tobacco use can induce aneuploidy within the oral epithelium, and in potentially malignant disorders, the epithelial cells change from diploid to an aneuploid pattern [85]. Aneuploidy is an indication either of an unbalanced number of chromosomes or unbalanced chromosomal regions resulting from deletions, duplications, amplifications or translocations [86]. Between 50% and 80% of oral SCC will have abnormal DNA content, and there is strong evidence to suggest that detecting such changes in leukoplakia and erythroplakia increases the risk of malignant transformation irrespective of the grade of dysplasia [87]. It is possible to combine DNA ploidy assessment with biomarker expression assessment such as Ki67 and p53 [88]. Van Zyl et al. [89] used high-resolution flow cytometry to determine the ploidy status of formalin-fixed tissues. Aneuploidy was found in 13% of mild, 31% of moderate and 54% of severe dysplasia cases. Such findings have been confirmed in earlier studies [87, 90, 91]. This difference was seen to be statistically significant. Sperandio et al. showed that combining DNA ploidy analysis with dysplasia grading can give a

higher predictive value than either technique alone [92]. The positive predictive value for malignant transformation by DNA aneuploidy was 38.5% and by severe dysplasia 39.5%. An alternative is the assessment of chromosomal instability by DNA image cytometry and FISH analysis which can be used for assessing lesions over time [93].

Optical Biopsy Adjuncts

The gold standard diagnostic assessment is biopsy and histological diagnosis. This can require several hospital visits which can lead to increased patient anxiety and increase cost both to the patient and the healthcare provider [94, 95]. Optical diagnostics can be useful in that they can provide an instantaneous assessment of tissue architecture and detect submucosal pathology. They have the potential to target biopsy tissue at the time of examination [96].

Chemiluminescence

Chemiluminescence works on the basis that there will be a change in the absorptive and reflective properties of light based on abnormal changes in metabolic and structural changes in tissues [97]. It was primarily used in the assessment of lesions in the cervical mucosa and was applied to oral SCC due to the similarities in clinical appearance [98]. Products commonly used include ViziLite®, ViziLite Plus®, VELscope™ and MicroLux™ and can be used in both primary and secondary care settings.

Three systems use a 60-s rinse in an acetic acid solution followed by inspection with a blue-white light source between 430 and 580 nm [97]. The light is emitted following flexion of a hand-held light stick which fractures resulting in a reaction between salicylic acid and hydrogen peroxide. This leads to a blue-white light being released for 10 min. The ViziLite Plus® uses toluidine blue to delineate lesions. MicroLux™ uses a battery-operated light source from a blue-white-emitting diode together with a fibre optic light guide. Rashid and Warnakulasuriya performed a meta-analysis which showed that there is variation in the sensitivity of the devices to detect dysplasia from 0% to 100%. The authors commented that the 100% sensitivity reported by some studies [99–101] is likely due to clinical findings. Other studies did not comment on sensitivity rates owing to the lack of comparison with histopathology. It was reported in one study that these devices may be unable to detect red lesions as they could only elicit a 50% response with erythroplakias [100]. This was confirmed in subsequent studies [93, 94] and suggests that chemiluminesence will more likely detect leukoplakia [96, 102]. One study [103] identified in the meta-analysis assessed MicroLux™. They reported a sensitivity rate of 77.8%, and the authors commented on the lack of discrimination between inflam-

matory, traumatic and malignant oral lesions. It has been suggested that the use of toluidine blue may provide an additive benefit. Two studies reported improvements in the specificity rate. Overall, despite improvements in visualisation, these systems do not significantly improve lesion detection, and further studies are needed to assess long-term follow-up [96]. To date, one meta-analysis [104] has assessed VELscope™. In the authors' assessment of 11 studies, high sensitivities were reported (92–100%) and improved sensitivity of detecting OPMD from 17% to 100%, but the major issue was the inability to distinguish between benign and OPMD lesions where half of the dysplasia cases were not detected [97]. The study overall advised that it did not improve sensitivity or specificity values and so should only be used by a specialist [104].

Optical Coherence Tomography (OCT)

OCT is a direct analogue of ultrasound using infrared light which records reflections below the surface to provide a cross-sectional image of the tissue. It can be used to assess both oral lesions and biopsy tissue. OCT can be used to monitor patients with oral dysplasia and enable early diagnosis, but variation can occur between observers [105]. OCT can detect architectural changes but unable to detect cytological atypia [106]. OCT has the potential to delineate surgical margins by finding an increase in the mean epithelial thickness and detecting architectural changes which is consistent in tumour-infiltrated tissue [107].

Microendoscopy

Microendoscopy allows for real-time diagnosis of tissues allowing for informed decisions to be made as to biopsy or resection margins [95]. It assesses the mucosal surface reducing the size of an incisional biopsy. The microendoscope is attached to a camera and scopes of varying size. The maximum magnification is 150×. In studies where comparisons were made between microendoscopy, paraffin sections and frozen sections, microendoscopy was able to detect dysplastic mucosa with a sensitivity of 95% and a specificity of 90%. This can be increased by further training to 98% and 92%, respectively [95].

Narrow Band Imaging (NBI)

NBI enhances tissue contrast by identifying superficial capillaries and neoangiogenesis with abnormal tissue by using a colour filter to narrow the bandwidth of spectral transmittance. This eliminates all wavelengths apart from narrow bands

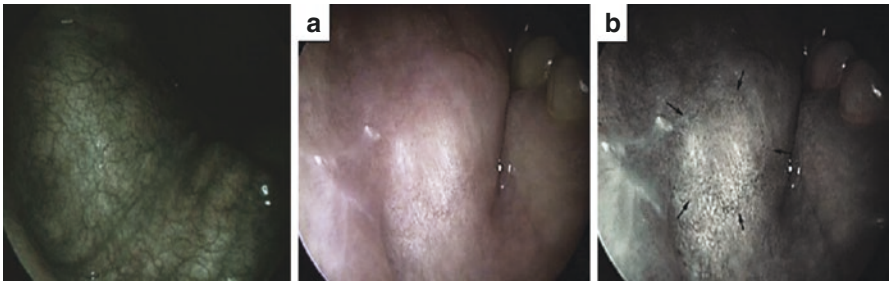

Fig. 10.2 Narrow-band imaging of normal oral mucosa showing clear branching vessels in the subepithelial layer which appear cyan, and the capillaries in the epithelial layer appear brown. The buccal mucosa in the middle and right of the figure (**a** and **b**) shows brownish dots on dilatation with weaving and differing shapes plus loss of the regular capillary arrangement (By kind permission of Professor Peter A. Brennan)

with central wavelengths of 415 and 580 nm (Fig. 10.2) [95, 108]. NBI does not require fluorescent dyes and is able to target suspicious vascular morphology, reducing the need for multiple biopsies [88]. The oral mucosa appears cyan and has clear branching vessels in the subepithelial layer. Abnormal vessels lose the regular capillary arrangement and appear brown on dilatation. The study was performed comparing normal white light examination with NBI in combination with high-definition television (HDTV) in 96 patients. Thirty-five patients were newly diagnosed and NBI identified 14 additional findings [109]. NBI can detect intracapillary loops which can indicate the increased severity of oral leukoplakia. Yang et al. performed a study comparing NBI with standard white light examination and found on NBI that 68.1% had squamous hyperplasia with hyperkeratosis, whereas 0.63% had high-grade dysplasia or invasive carcinoma. The study confirmed that there was a relationship between disease severity and angiogenesis and destruction in the intracapillary loops [110].

Fluorescence

Tumour-specific intrinsic fluorescence arises from tumour-induced morphogenic and biochemical alterations which change the way tissues interact with light. Systems generally emit violet light (400 nm) through LED systems [95]. There is reduced fluorescence in abnormal tissue, whereas normal tissue is apple-green and at a larger wavelength (450–500 nm). Autofluorescence which is used to delineate surgical margins in oral tissues is based on the variety of fluorophores found in the oral cavity and has been suggested that loss of fluorescence in neoplastic tissue is due to reduced collagen cross-links in stromal tissue [95]. It has been shown that fluorescence is useful in assessing whether the lesion extends beyond what is clinically observed [111]. The majority of studies have only assessed leukoplakia [112, 113]. Early studies [114] were able to show in 44 patients a specificity and

sensitivity of 98% and 100%, respectively. This has been confirmed in later studies [115] where there was an increase in the detection rate of OPMD, and it has been suggested that it may enhance clinical examination [1]. However, there is variation in specificity when using fluorescence (50% versus 60%) [115]. Recent studies showed that there was an increase in accuracy for detecting OPMD lesions compared to white light examination from 46.66% to 53.33%. Visualisation improved by using fluorescence when less experienced hands were assessing lesions [116].

Conclusion

Biopsy with histopathological analysis remains the gold standard for assessing OPMD lesions. However, it is not useful as a predictive tool for oral dysplasia. Also due to its invasive nature and risk of needing multiple biopsies, it means that there remains the potential of optical biopsy adjuncts as part of management. The use of biomarkers, DNA ploidy analysis and immunohistochemical panels plus adjuncts may make effective clinical tools for assessing malignant transformation. Although the risk of malignant transformation is low, there needs to be regular clinical assessment prior to biopsy together with multicentre randomised controlled trials.

References

1. Field EA, McCarthy CE, Ho MW, et al. The management of oral epithelial dysplasia: the Liverpool algorithm. Oral Oncol. 2015;51:883–7.
2. Green B, Mendes RA, Van der Valk R, Brennan PA. Novel anticoagulants- an update on the latest developments and management for clinicians treating patients on these drugs. J Oral Pathol Med. 2016;45:551–6.
3. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45:317–23.
4. Kujan O, Oliver RJ, Khattab A, et al. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006;42:987–93.
5. Nankivell P, Williams H, Matthews P, et al. The binary oral dysplasia grading system: validity testing and suggested improvement. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115:87–94.
6. Dost F, Le Cao K, Ford PJ, et al. Malignant transformation of oral epithelial dysplasia: a real-world evaluation of histopathologic grading. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117:343–52.
7. Bagan JV, Jiminez Y, Sanchis JM, et al. Proliferative verrucous leukoplakia: high incidence of gingival squamous cell carcinoma. J Oral Pathol Med. 2003;32:379–82.
8. van der Waal I, Reichart PA. Oral proliferative verrucous leukoplakia revisited. Oral Oncol. 2008;44:719–21.
9. Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. J Oral Pathol Med. 2016;45:155–66.
10. Ho MW, Risk JM, Woolgar JA, et al. The clinical determinants of malignant transformation of oral epithelial dysplasia. Oral Oncol. 2012;48:969–76.

11. Ho MW, Field EA, Field JK, et al. Outcomes of oral squamous cell carcinoma arising from oral epithelial dysplasia: rationale for monitoring premalignant lesions in a multidisciplinary clinic. Br J Oral Maxillofac Surg. 2013;51:594–9.
12. Speight PM. Update on oral epithelial dysplasia and progression to cancer. Head Neck Pathol. 2007;1:61–6.
13. van der Waal I, Schepman KP, van der Meij EH, Smeele LE. Oral leukoplakia: a clinicopathological review. Oral Oncol. 1997;33:291–301.
14. Thomson PJ, McCaul JA, Ridout F, Hutchinson IL. To treat…or not to treat? Clinicians view on the management of oral potentially malignant disorders. Br J Oral Maxillofac Surg. 2015;53:1027–31.
15. Kanatas AN, Fisher SE, Lowe D, et al. The configurations of clinics and the use of biopsy and photography in oral premalignancy: a survey of consultants of the British Association of Oral & Maxillofacial Surgeons. Br J Oral Maxillofac Surg. 2011;49:99–105.
16. Kumar A, Cascarini L, McCaul JA, et al. How should we manage oral leukoplakia? Br J Oral Maxillofac Surg. 2013;51:377–83.
17. Mehanna HM, Rattay T, Smith J, McConkey CC. Treatment and follow-up of oral dysplasia-a systematic review and meta-analysis. Head Neck. 2009;31:1600–9.
18. Balasundaram I, Payne KF, Al-Hadad I, et al. is there any benefit in surgery for potentially malignant disorders of the oral cavity? J Oral Pathol Med. 2014;43:239–44.
19. Howlett DC, Triantafyllou A. Evaluation: fine needle aspiration cytology, ultrasound-guided core biopsy and open biopsy techniques. Adv Otorhinolaryngol. 2016;78:39–45.
20. Triantafyllou A, Hunt JL, Devaney KO, Ferlito A. A perspective of comparative salivary and breast pathology: Part I: microstructural aspects, adaptations and cellular events. Eur Arch Otorhinolaryngol. 2014;271:647–63.
21. Gillani M, Akhtar F, Ali Z, et al. Diagnostic accuracy, sensitivity, specificity and positive predictive value of fine needle aspiration cytology (FNAC) in intra oral tumours. Asian Pac J Cancer Prev. 2012;13:3611–5.
22. Gandhi S, Lata J, Gandhi N. Fine needle aspiration cytology: a diagnostic aid for oral lesions. J Oral Maxillofac Surg. 2011;69:1668–77.
23. Wagner JM, Conrad RD, Cannon TY, Alleman AM. Ultrasound-guided transcutaneous needle biopsy of the base of tongue and floor of mouth from a submental approach. J Ultrasound Med. 2016;35:1009–13.
24. Mehrotra R. The role of cytology in oral lesions: a review of recent improvements. Diagn Cytopathol. 2012;40:73–83.
25. Gupta S, Shah JS, Parikh S, et al. Clinical correlative study on early detection of oral cancer and precancerous lesions by modified oral brush biopsy and cytology followed by histopathology. J Canc Res Ther. 2014;10:232–8.
26. Trakroo A, Sunil MK, Trivedi A, et al. Efficacy of oral brush biopsy without computer-assisted analysis in oral premalignant and malignant lesions: a study. J Int Oral Health. 2015;7:33–8.
27. Babshet M, Nandimath K, Pervatikar S, Naikmasur V. Efficacy of oral brush cytology in the evaluation of the oral premalignant and malignant lesions. J Cytol. 2011;28:16–172.
28. Ye X, Zhang J, Tan Y, et al. Meta-analysis of two computer-assisted screening methods for diagnosing oral precancer and cancer. Oral Oncol. 2015;1:966–75.
29. Navone R, Pentenero M, Rostan I, et al. Oral potentially malignant lesions: first-level microhistological diagnosis from tissue fragments sampled in liquid-based diagnostic cytology. J Oral Pathol Med. 2008;37:358–63.
30. Pentenero M, Marino R, Tempia Valenta G, et al. Microbiopsy a novel sampling technique to early detect dysplastic/malignant alterations in oral mucosal lesions: practicability by general dentists. J Oral Pathol Med. 2014;43:435–40.
31. Haldar S, Sinnott JD, Tekeli KM, et al. Biopsy of parotid masses: review of current techniques. World J Radiol. 2016;28:501–5.
32. Shah KS, Ethunandan M. Tumour seeding after fine-needle aspiration and core biopsy of the head and neck--a systematic review. Br J Oral Maxillofac Surg. 2016;54:260–5.

33. Howlett DC, Skelton E, Moody AB. Establishing an accurate diagnosis of a parotid lump: evaluation of the current biopsy methods - fine needle aspiration cytology, ultrasound-guided core biopsy, and intraoperative frozen section. Br J Oral Maxillofac Surg. 2015;53:580–3.
34. Skelton E, Jewison A, Okpaluba C, et al. Image-guided core needle biopsy in the diagnosis of malignant lymphoma. Eur J Surg Oncol. 2015;41:852–8.
35. Pfeiffer J, Maier W, Boedeker CC, Ridder GJ. Transmucosal core needle biopsy: a novel diagnostic approach to oral and oropharyngeal lesions. J Oral Maxillofac Surg. 2014;72:1594–600.
36. Ahn D, Roh JH, Kim JK. Ultrasound-guided core needle biopsy for head and neck mass lesions in patients undergoing antiplatelet or anticoagulation therapy: a preliminary report. J Ultrasound Med. 2017;36:1339–46.
37. Novoa E, Gurtler N, Amoux A, Kraft M. Role of ultrasound-guided core-needle biopsy in the assessment of head and neck lesions: a meta-analysis and systematic review of the literature. Head Neck. 2012;34:1497–503.
38. Allin D, David S, Jacob A, et al. Use of core biopsy in diagnosing cervical lymphadenopathy: a viable alternative to surgical excisional biopsy of lymph nodes? Ann R Coll Surg Engl. 2017;99:242–4.
39. Goodson ML, Sloan P, Robinson CM, et al. Oral precursor lesions and malignant transformation--who, where, what, and when? Br J Oral Maxillofac Surg. 2015;53:831–5.
40. Pentenero M, Carrozzo M, Pagano M, et al. Oral mucosal dysplastic lesions and early squamous cell carcinomas: underdiagnosis from incisional biopsy. Oral Dis. 2003;9:68–72.
41. Lee JJ, Hung HC, Cheng SJ, et al. Factors associated with underdiagnosis from incisional biopsy of oral leukoplakic lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104:217–25.
42. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37:1–10.
43. Jeong WJ, Paik JH, Cho SW, et al. Excisional biopsy for management of tongue leukoplakia. J Oral Pathol Med. 2012;41:384–8.
44. Kerawala CJ. Incisional biopsy: reducing artefact. Br J Oral Maxillofac Surg. 1995;33:396.
45. Dhanda J, Uppal N, Chowlia H, et al. Features and prognostic utility of biopsy in oral squamous cell carcinoma. Head Neck. 2012;38:E1857–62.
46. Shah N, Ray JG, Kundu S, Sardana D. Surgical management of chronic hyperplastic candidiasis refractory to systemic antifungal treatment. J Lab Phys. 2017;9:136–9.
47. Sitheeque MA, Samaranayake LP. Chronic hyperplastic candidosis/candidiasis (candidal leukoplakia). Crit Rev Oral Biol Med. 2003;14:253–67.
48. Krishnan PA. Fungal infections of the oral mucosa. Indian J Dent Res. 2012;23:650–9.
49. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. J Oral Microbiol. 2011;3:5771.
50. Shin ES, Chung SC, Kim YK, et al. The relationship between oral *Candida* carriage and the secretor status of blood group antigens in saliva. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96:48–53.
51. Field EA, Field JK, Martin MV. Does *Candida* have a role in oral epithelial neoplasia? J Med Vet Mycol. 1989;27:277–94.
52. Gainza-Ciraugui ML, Nieminen MT, Novak Frazer L, et al. Production of carcinogenic acetaldehyde by Candida albicans from patients with potentially malignant oral mucosal disorders. J Oral Pathol Med. 2013;42:243–9.
53. McCullough M, Jaber M, Barrett AW, et al. Oral yeast carriage correlates with presence of oral epithelial dysplasia. Oral Oncol. 2002;38:391–3.
54. Pontes HA, Paiva HB, de Freitas Silva BS, et al. Oral candidiasis mimicking an oral squamous cell carcinoma: report of a case. Gerodontology. 2012;29:70–4.
55. Abdulmajeed AA, Farah CS. Can immunohistochemistry serve as an alternative to subjective histopathological diagnosis of oral epithelial dysplasia? Biomark Cancer. 2013;10:49–60.

56. Bienk Dias K, Pereira Costa Flores A, Gaiger Oliveira M, et al. Predictive value of p63, ki-67, and survivin expression in oral leukoplakia: a tissue microarray study. Microsc Res Tech. 2017;80:845.
57. Pigatti FM, Taveira LA, Soares CT. Immunohistochemical expression of Bcl-2 and Ki-67 in oral lichen planus and leukoplakia with different degrees of dysplasia. Int J Dermatol. 2015;54:150–5.
58. Acay RR, Felizzola CR, de Araujo N, de Sousa SO. Evaluation of proliferative potential in oral lichen planus and oral lichenoid lesions using immunohistochemical expression of p53 and Ki67. Oral Oncol. 2006;42:475–80.
59. Dionne KR, Warnakulasuriya S, Zain RB, Cheong SK. Potentially malignant disorders of the oral cavity. Int J Cancer. 2015;136:503–15.
60. Mattila R, Alanen K, Syrjanen S. Immunohistochemical study on topoisomerase IIalpha, Ki-67 and cytokeratin-19 in oral lichen planus lesions. Arch Dermatol Res. 2007;298:381–8.
61. Sudha VM, Hemavathy S. Role of bcl-2 oncoprotein in oral potentially malignant disorders and squamous cell carcinoma: an immunohistochemical study. Indian J Dent Res. 2011;22:520–5.
62. Garewal J, Garewal R, Sircar R. Expression of Bcl-2 and MIB-1 markers in oral squamous cell carcinoma (OSCC) - a comparative study. J Clin Diagn Res. 2014;8:QC01–4.
63. Silva AD, Maraschin BJ, Laureano NK, et al. Expression of E-cadherin and involucrin in leukoplakia and oral cancer: an immunocytochemical and immunohistochemical study. Braz Oral Res. 2017;6:e19.
64. Du Y, Li H. Expression of E-cadherin in oral lichen planus. Exp Ther Med. 2015;10:1544–8.
65. Nguyen CT, Okamura T, Morita KI, et al. LAMC2 is a predictive marker for the malignant progression of leukoplakia. J Oral Pathol Med. 2017;46:223–31.
66. Chaudhari NT, Tupkari JV, Joy T, Ahire MS. Human MutL homolog 1 immunoexpression in oral leukoplakia and oral squamous cell carcinoma: a prospective study in Indian population. J Oral Maxillofac Pathol. 2016;20:453–61.
67. Bhattacharya A, Roy R, Snijders AM, et al. Two distinct routes to oral cancer differing in genome instability and risk for cervical node metastases. Clin Cancer Res. 2011;17:7024–34.
68. Bhosale PG, Cristea S, Ambatipudi S, et al. Chromosomal alterations and gene expression changes associated with the progression of leukoplakia to advanced gingivobuccal cancer. Transl Oncol. 2017;10:396–409.
69. Lee JJ, Hong WK, Hittleman WN, et al. Predicting cancer development in oral leukoplakia: ten years of translational research. Clin Cancer Res. 2000;6:1702–10.
70. Partridge M, Pateromichelakis S, Phillps E, et al. A case-control study confirms that microsatellite assay can identify patients at risk of developing oral squamous cell carcinoma within a field of cancerisation. Cancer Res. 2000;60:3893–8.
71. Rosin MP, Cheng X, Poh C, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res. 2000;6:357–62.
72. Rousseau A, Lim MS, Lim Z, Jordan RC. Frequent cyclin D1 gene amplification and protein overexpression in oral epithelial dysplasias. Oral Oncol. 2001;37:268–75.
73. Poh CF, Zhu Y, Chen E, et al. Unique FISH patterns associated with cancer progression of oral dysplasia. J Dent Res. 2012;91:52–7.
74. Raju B, Mehrotra R, Oijordsbakken G, et al. Expression of p53, cyclin D1 and Ki-67 in pre-malignant and malignant oral lesions: association with clinicopathological parameters. Anticancer Res. 2005;25:4699–706.
75. Inoue H, Miyazaki Y, Kikuchi K, et al. Podoplanin promotes cell migration via the EGF-Src-Cas pathway in oral squamous cell carcinoma cell lines. J Oral Sci. 2012;54:241–50.
76. D'Souza B, Nayak R, Kotrashetti VS. Immunohistochemical expression of podoplanin in clinical variants of oral leukoplakia and its correlation with epithelial dysplasia. Appl Immunohistochem Mol Morphol. 2018;26:132.

77. Saintigny P, El-Naggar AK, Papadimitrakopoulou V, et al. DeltaNp63 overexpression, alone and in combination with other biomarkers, predicts the development of oral cancer in patients with leukoplakia. Clin Cancer Res. 2009;15:6284–91.
78. Feng JQ, Mi JG, Wu L, et al. Expression of podoplanin and ABCG2 in oral erythroplakia correlate with oral cancer development. Oral Oncol. 2012;48:848–52.
79. Kreppel M, Kreppel B, Drebber U, et al. Podoplanin expression in oral leukoplakia: prognostic value and clinicopathological implications. Oral Dis. 2012;18:692–9.
80. Habiba U, Hida K, Kitamura T, et al. ALDH1 and podoplanin expression patterns predict the risk of malignant transformation in oral leukoplakia. Oncol Lett. 2017;13:321–8.
81. Shaw R. The epigenetics of oral cancer. Int J Oral Maxillofac Surg. 2006;35:101–8.
82. Alazari NA, Sperandio M, Odell EW, et al. Meta-analysis of the predictive value of DNA aneuploidy in malignant transformation of oral potentially malignant disorders. J Oral Pathol Med. 2018;47:97–103.
83. Hall GL, Shaw RJ, Field EA, et al. p16 Promoter methylation is a potential predictor of malignant transformation in oral epithelial dysplasia. Cancer Epidemiol Biomarkers Prev. 2008;17:2174–9.
84. Abe M, Yamashita S, Mori Y, et al. High-risk oral leukoplakia is associated with aberrant promoter methylation of multiple genes. BMC Cancer. 2016;16:350.
85. Souto GR, Caliari MV, Lins CE, et al. Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. J Oral Pathol Med. 2010;39:605–10.
86. Giaretti W, Monteghirfo S, Pentenero M, et al. Chromosomal instability, DNA index, dysplasia, and subsite in oral premalignancy as intermediate endpoints of risk of cancer. Cancer Epidemiol Biomarkers Prev. 2013;22:1133–41.
87. Bremmer JF, Brakenhoff RH, Broeckaert MA, et al. Prognostic value of DNA ploidy status in patients with oral leukoplakia. Oral Oncol. 2011;47:956–60.
88. Fleskens SJ, Takes RP, Otte-Holler I, et al. Simultaneous assessment of DNA ploidy and biomarker expression in paraffin-embedded tissue sections. Histopathology. 2010;57:14–26.
89. van Zyl AW, van Heerden MB, Langenegger E, van Heerden WF. Correlation between dysplasia and ploidy status in oral leukoplakia. Head Neck Pathol. 2012;6:322–7.
90. Torres-Rendon A, Stewart R, Craig GT, et al. DNA ploidy analysis by image cytometry helps to identify oral epithelial dysplasias with a high risk of malignant progression. Oral Oncol. 2009;45:468–73.
91. Bradley G, Odell EW, Raphael S, et al. Abnormal DNA content in oral epithelial dysplasia is associated with increased risk of progression to carcinoma. Br J Cancer. 2010;103:1432–42.
92. Sperandio M, Brown AL, Lock C, et al. Predictive value of dysplasia grading and DNA ploidy in malignant transformation of oral potentially malignant disorders. Cancer Prev Res (Phila). 2013;6:822–31.
93. Siebers TJ, Bergshoeff VE, Otte-Holler I, et al. Chromosome instability predicts the progression of premalignant oral lesions. Oral Oncol. 2013;49:1121–8.
94. Adegun OK, Tomlins PH, Hagi-Pavli PE, et al. Quantitative analysis of optical coherence tomography and histopathology images of normal and dysplastic oral mucosal tissues. Lasers Sci Med. 2012;27:795–804.
95. Green B, Tsiroyannis C, Brennan PA. Optical diagnostic systems for assessing head and neck lesions. Oral Dis. 2016;22:180–4.
96. Rashid A, Warnakulasuriya S. The use of light-based (optical) detection systems as adjuncts in the detection of oral cancer and oral potentially malignant disorders: a systematic review. J Oral Pathol Med. 2015;44:307–28.
97. Huber MA, Bsoul SA, Terezhalmy GT. Acetic acid wash and chemiluminescent illumination as an adjunct to conventional oral soft tissue examination for the detection of dysplasia: a pilot study. Quintessence Int. 2004;35:378–84.
98. Ram S, Siar CH. Chemiluminescence as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions. Int J Oral Maxillofac Surg. 2005;34:521–7.

99. Farah CS, McCollough MJ. A pilot case control study on the efficacy of acetic acid wash and chemiluminescent illumination (ViziLite) in the visualisation of oral mucosal white lesions. Oral Oncol. 2007;43:820–4.
100. Awan KW, Morgan PR, Warnakulasuriya S. Utility of chemiluminescence (ViziLite™) in the detection of oral potentially malignant disorders and benign keratoses. J Oral Pathol Med. 2011;40:541–4.
101. Epstein JB, Gorsky M, Lonky S, et al. The efficacy of oral lumenoscopy (ViziLite) in visualizing oral mucosal lesions. Spec Care Dent. 2006;26:171–4.
102. Kerr AR, Sirois DA, Epstein JB. Clinical evaluation of chemiluminescent lighting: an adjunct for oral mucosal examinations. J Clin Dent. 2006;17:59–63.
103. McIntosh L, McCollough MJ, Farah CS. The assessment of diffused light illumination and acetic acid rinse (Microlux/DL) in the visualisation of oral mucosal lesions. Oral Oncol. 2009;45:e227–31.
104. Awan KH, Patil S. Efficacy of autofluorescence imaging as an adjunctive technique for examination and detection of oral potentially malignant disorders: a systematic review. J Contemp Dent Pract. 2015;16:744–9.
105. Mehrotra R, Singh M, Thomas S, et al. A cross-sectional study evaluating chemiluminescence and autofluorescence in the detection of clinically innocuous precancerous and cancerous oral lesions. J Am Dent Assoc. 2010;141:151–6.
106. Hamdoon Z, Jerjes W, McKenzie G, et al. Optical coherence tomography in the assessment of oral squamous cell carcinoma resection margins. Photodiagnosis Photodyn Ther. 2016;13:211–7.
107. Jerjes W, Upile T, Conn B, et al. In vitro examination of suspicious oral lesions using optical coherence tomography. Br J Oral Maxillofac Surg. 2010;48:18–25.
108. Tan NC, Herd MK, Brennan PA, Puxeddu R. The role of narrowband imaging in early detection of head and neck cancer. Br J Oral Maxillofac Surg. 2012;50:132–6.
109. Piazza C, Cocco D, Del Bon F, et al. Narrow band imaging and high definition television in the endoscopic evaluation of upper aero-digestive tract cancer. Acta Otorhinolaryngol Ital. 2011;31:70–5.
110. Yang SW, Lee YS, Chang LC, et al. Light sources used in evaluating oral leukoplakia: broadband white light versus narrowband imaging. Int J Oral Maxillofac Surg. 2013;42:693–701.
111. Elvers D, Braunschweig T, Hilgers RD, et al. Margins of oral leukoplakia: autofluorescence and histopathology. Br J Oral Maxillofac Surg. 2015;53:164–9.
112. Farah CS, McIntosh L, Georgiou A, McCollough MJ. Efficacy of tissue autofluorescence imaging (VELScope) in the visualization of oral mucosal lesions. Head Neck. 2012;34:856–62.
113. Simonato LE, Tomo S, Miyahara GI, et al. Fluorescence visualization efficacy for detecting oral lesions more prone to be dysplastic and potentially malignant disorders: a pilot study. Photodiagnosis Photodyn Ther. 2017;17:1–4.
114. Lane PM, Gilhuly T, Whitehead P, et al. Simple device for the direct visualization of oral-cavity tissue fluorescence. J Biomed Opt. 2006;11:024006.
115. Huff K, Stark PC, Solomon LW. Sensitivity of direct tissue fluorescence visualization in screening for oral premalignant lesions in general practice. Gen Dent. 2009;57:34–8.
116. Klatt J, Gerich GE, Grobe A, et al. Fractal dimension of time-resolved autofluorescence discriminates tumour from healthy tissues in the oral cavity. J Craniomaxillofac Surg. 2014;42:852–4.

Chapter 11
Management of Premalignant Disease of the Oral Mucosa

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Introduction

Oral potentially malignant disorders (OPMDs) have been defined as 'a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart' [1, 2]. A number of lesions and conditions are included under the umbrella of OPMD including (1) leukoplakia, (2) erythroplakia, (3) oral submucous fibrosis, (4) palatal lesions in reverse smokers, (5) oral lichen planus, (6) discoid lupus erythematosus and (7) actinic cheilitis [3]. In addition, rare inherited conditions, such as xeroderma pigmentosum and Fanconi's anaemia, carry an increased incidence of oral cancer. Immunodeficiency due to the prolonged use of immunosuppressive drugs or due to an underlying HIV infection may increase risk, and oral cancer has also been reported in patients suffering from chronic graft versus host disease (GVHD) after haematopoietic stem cell transplantation. A risk assessment tool for head and neck cancer is shown in Fig. 11.1.

Of these conditions, leukoplakia and erythroplakia are considered the most likely to undergo malignant transformation. There are however a number of possible outcomes besides malignant transformation [4]. The lesion may remain unchanged, or it may increase or decrease in size or completely resolve. An assessment of likelihood of progression is based on clinical parameters, the aetiology of the lesion and risk factors.

The perceived progression of oral lichen planus (OLP) to OSCC has generated a long-standing controversy about its malignant potential. The erosive and atrophic forms of OLP are more prevalent among patients who have developed OSCC in OLP lesions [5, 6]. OLP is a common condition, but there are no precise clinical, histological or molecular predictors of malignant potential. It has been suggested

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PREDICTIVE RISK ASSESSMENT OF HEAD AND NECK CANCER

1.	**Your Age**						
	20s	30s	40s	50s	60s	70s	80s
2.	**Your Gender**						
	FEMALE						MALE
3.	**Smoke cigarettes or other forms of tobacco such as bidis, cigars, cigarillos, little cigars, kreteks, pipes, or hookas**						
	NO			Former Smoker			YES
4.	**Use areca nut or betel quid on a regular basis**						
	NO			Former User			YES
5.	**Chew tobacco, use spit tobacco (dip or oral dissolvable tobacco products), sniff or inhale snuff**						
	NO			Former Tobacco User			YES
6.	**Drink alcohol frequently and consume large amounts**						
	NO			Former Drinker			YES
7.	**Human Papillomavirus (HPV) vaccine**						
	NO			I have not had HPV vaccination (age above 26)			YES
	NO			I started my HPV vaccines, but did not get all 3 shots (ages 9-26)			YES
	NO			I have not completed my HPV vaccine series of 3 shots within a 6-month period (ages 9-26)			YES
8.	**Sexual behaviour**						
	NO			I have oral sex with several partners			YES
	NO			My partner(s) engages in sex with several others			YES
9.	**Family history of cancer**						
	NO			I have a family history of head or neck cancer including mouth, lips, nose, or throat			YES
	NO			I have a personal history of cervical cancer (females only)			YES
	NO			I have a personal history of prostate or breast cancer			YES
	NO			I have Fanconi anaemia, Ataxia-telangiectasia, Xeroderma pigmentosum, Bloom's syndrome, Dyskeratosis congenita, or Li-Fraumeni syndrome			YES
	NO			I suffer from an immunosuppressive disorder or condition			YES
10.	**Other lifestyle choices**						
	NO			I do not eat a diet rich in vegetables and fruits			YES
	NO			I eat salt preserved foods regularly (examples: salted fish, salted meat)			YES
	NO			I work with wood, sawdust, asbestos, toxic fumes			YES
	NO			I do not protect my lips from the sun's ultraviolet (UV) rays with balm that has sun protective factor			YES
	NO			I have very poor oral hygiene			YES
	NO			I use alcoholic mouthwashes on a regular basis			YES
11.	**Head and neck cancer symptoms**						
	NO			White or red patch or ulcer on the gums, tongue or lining of the mouth			YES
	NO			Painful or difficult to swallow; feeling of something caught in the throat			YES
	NO			Mass or lump in the neck; pain or swelling in the face, chin or neck			YES
	NO			Sore throat or a cough that doesn't go away			YES
	NO			Trouble breathing or speaking; hoarseness or a change in voice			YES
	NO			Glands or lymph nodes in the neck are enlarged			YES

This list of head and neck cancer risk factors and symptoms is derived from information published by the National Cancer Institute (NCI), the American Dental Association, the Oral Cancer Foundation, the Georgia Cancer Centre Augusta University, Cancer Australia, and published literature. Some of the symptoms may also relate to other illnesses or conditions.

Fig. 11.1 Predictive risk assessment of head and neck cancer (Image adapted and used with permission from C. S. Farah et al. (eds.), Contemporary Oral Medicine, Springer Nature Switzerland AG 2019)

that structured follow-up and reporting strategies may help determine the real risk associated with malignant transformation in OLP [7].

The frequency of malignant transformation for OLP has been reported to be between 0.4% and 5% over an observation time period of 0.5–20 years by some authors [8, 9]. Other reviews with a follow-up of over 2 years applying strict diagnostic criteria reported a malignant transformation rate of 0–2% [10].

No accurate data is available on the malignant transformation rates of actinic cheilitis, although they have been reported at 16.9% in one study [11]. It is generally accepted that the majority of cases of squamous cell carcinoma of the lower lip are preceded by actinic cheilitis.

Clinicians and patients are faced with a number of questions when confronted with premalignant disease. These include (1) What lesions require treatment? (2) What is the benefit to the patient in treating lesions? (3) What risks are involved in treating or abstaining from treatment? (4) What are the challenges involved in treatment? (5) What is the cost of treatment and regular follow-up? Risk assessment in order to address these questions involves factors related to the lesion(s) and factors related to the patient. The malignant transformation of OPMDs to oral squamous cell carcinoma (OSCC) has been studied in many different populations, and this evidence has been reviewed by Napier and Speight [12].

The evaluation of any oral mucosal lesion should include an assessment of the risk of malignant transformation based on factors related to the patient and the nature of the lesion.

Management Rationales of Oral Potentially Malignant Disorders

When a diagnosis of an OPMD is established, a decision needs to be made whether to actively treat the lesion or condition in question or to simply observe it. The immediately apparent problem with observation of an OPMD is that, by the very definition of these disorders, in some cases, this simply leads to the supervised development of cancer, perhaps with early detection being the sole conciliation, and even this is not always achievable. Perceived time pressures, logistical appointment attendance barriers, general lack of oral cancer knowledge, absence of pain and unwillingness to discover disease constitute some of the documented barriers responsible for low uptake of oral mucosal screening programmes—even in the absence of financial constraints [13]. Conversely, the option of treatment—the medical or surgical management of a patient—be it definitive or palliative, implies that treatment is available, that it is possible and, in the current age, that it is evidence based. Numerous disease and patient characteristics impact on the decision to initiate treatment or recommend surveillance.

The main reason for treating OPMDs is to reduce (and ideally eliminate) the risk of development of oral squamous cell carcinoma—a disease, which, on average, is

fatal in half of the individuals affected [14, 15]. Furthermore, the treatment of the carcinoma is complex and frequently associated with significant morbidity, leaving those who survive with a considerably compromised quality of life [16, 17]. The affected individual may be left with undisguisable deformity and/or significant loss or impairment of some of the most basic bodily functions. Resultant dysgeusia, dysphagia, dysphonia, loss of dentition, weight loss and malnutrition, trismus and neuropathic pain are common treatment consequences, often leading to some degree of social isolation. It is therefore not surprising that a recent US-based study [18] demonstrated that patients with oral and oropharyngeal squamous cell carcinoma were eight times more likely to commit suicide during the first year after cancer diagnosis, compared to the general US population. The individual and social economic burden associated with the treatment of oral SCC is also substantial. This stems from the complex nature of oral carcinoma treatment, which focuses not only on cure but also on organ preservation, often necessitating the utilisation of multimodality treatment and multidisciplinary expertise. The significant morbidity which often accompanies disease survival subsequently demands the ongoing utilisation of healthcare resources, further escalating the cost of care.

While the prevention of oral cancer and its sequelae is the obvious reason for treating OPMD, fortunately, for many of those afflicted, the outlook is less morbid. Nonetheless, although only some OPMDs undergo malignant transformation, the disorders have the capacity to adversely impact on life quality in other ways [19]. For example, OPMDs, by their nature, can create a situation of a life in limbo, potentially leading to significant psychological distress. Different kinds of distress have been associated with limbo situations such as anxiety, worry, dread and despair [20]. Anxiety—a state of unpleasant feelings when confronted with a specific situation, demand or threat—is a very common problem in patients diagnosed with cancer [21]. High levels of anxiety have also been reported to be associated with OPMDs, although this is a largely unexplored area [21]. Anxiety can in turn adversely affect the individual's ability to cope with seeking treatment, which may lead to diagnostic delay [21]. Furthermore, all OPMDs are capable of producing pain or discomfort [19], and some disorders, such as OSMF, can in themselves be severely disabling. Over time OSMF, for example, leads to worsening limitation of mouth opening and tongue protrusion, causing difficulty in eating, swallowing and phonation, eventually leaving individuals considerably handicapped physically and psychologically [22]. Halting disease progression in this instance is therefore also an important treatment aim [23].

Interestingly, despite the fact that OPMDs are common, chronic and potentially disabling, at present, the literature pertaining to the specific impacts of OPMDs on the individual's quality of life is limited [19]. Most studies to date have focused on OLP [24–36], and many have demonstrated compromised life quality [25–29, 31, 32, 35, 36], strongly influenced by the presence of symptoms. Improvements in life quality have been demonstrated in OLP patients with treatment [26, 27, 29, 32]. Only a few studies [35, 37, 38] examined the quality of life in patients with other OPMDs, namely, oral cGVHD, OSMF and oral leukoplakia, demonstrating a decreased life quality in patients with cGVHD and OSMF [35, 37, 38]. Karbach

et al. [35] in a small study failed to demonstrate a significant difference in quality of life scores between individuals with OLP, leukoplakia and oral SCC. While the adverse impacts of OPMDs on life quality may seem obvious, a recently published systematic review examining the quality of life in patients with OPMDs concluded that, at present, there is no strong evidence that individuals with OPMDs have a poorer life quality compared to healthy controls [19]. This somewhat surprising result has been attributed to the low quality of studies conducted to date as most focus only on OLP, rarely include comparisons with healthy controls and at times utilise quality of life instruments of questionable validity [19].

Another argument in favour of treating OPMDs pertains to the role that treatment potentially plays in helping identify disease progression and aiding follow-up. OPMDs can mimic early oral SCC in their clinical presentation. Treatment, and more specifically the lack of response to treatment, of localised painful, erosive and/or ulcerated lesions, especially those associated with widespread oral mucosal disease such as OLP, oral DLE and oral cGVHD, may help to identify such lesions as sites undergoing malignant transformation and draw the clinician's attention to areas that require further investigation [39]. In cases of OPMD such as leukoplakia and erythroplakia where excision may be possible, complete removal of the lesion streamlines the process of surveillance. When compared to the act of ascertaining whether an existing lesion has changed with time, establishing the mere presence or absence of an OPMD is a much simpler process [40].

While it may appear that treatment is the obvious and ethical choice for all OPMDs, the challenges that clinicians face is that OPMDs encompass a very diverse group of lesions and conditions. The aetiology of many OPMDs is poorly or incompletely understood, and currently there is no proven curative treatment for any OPMD. In some situations, therefore, it can be argued that the currently available treatment options are not necessarily the best course of action to undertake. For example, in the case of OLP, where at present there is no curative treatment and no established predictive factors for malignant transformation, it is not believed possible to prevent future cancer development. The main management objectives are symptom control and early cancer detection. Thus, the asymptomatic, exclusively reticular and plaque-like forms of OLP do not benefit from active treatment, and, in those situations, follow-up only is recommended [41]. As is the case with OLP, oral DLE exhibits a prolonged clinical course which can persist for many years, despite various treatments, and follow-up only is recommended for asymptomatic, non-ulcerated lesions [42].

Reasons to treat any OPMD are clearly influenced by many factors. Despite the general recommendation that, ideally, all leukoplakias and erythroplakias be surgically treated [43], surgical intervention may not always be possible, particularly in the case of extensive or diffuse lesions or when the affected individual's comorbidities place them at excessive surgical risk. It may also not be an acceptable treatment option to the affected, asymptomatic individual, recognising that at present there is no definitive scientific evidence that any form of treatment truly prevents future cancer development nor is malignant transformation of any OPMD an inevitable outcome [16]. Close surveillance is then the only option [16] although this is reliant

on patient motivation and continued attendance for review, with added financial burden on the health system, where monitoring of patients may also include added investigations including multiple biopsies over time.

While numerous patient and lesion attributes have been associated with increased risk of malignant transformation of OPMDs, it is still not possible to predict, on an individual basis, which lesions will progress to malignancy. A 2016 systematic review of observational studies aiming to ascertain important risk factors for malignant transformation of oral leukoplakia—the most common OPMD—identified the grade of dysplasia, advanced age, female gender, lesion size greater than 200 mm^2 and non-homogenous type as the most important determinants of the malignant potential of this disorder [44].

Given the current level of knowledge and the potentially lethal nature of OSCC, ideally all leukoplakias (especially non-homogenous) and erythroplakias should be surgically treated and in particular those lesions exhibiting any grade of dysplasia [40, 43, 45, 46]. Where the decision to proceed with treatment is not a straightforward one, consideration of the risk factors below may help clinicians and patients in that decision-making process.

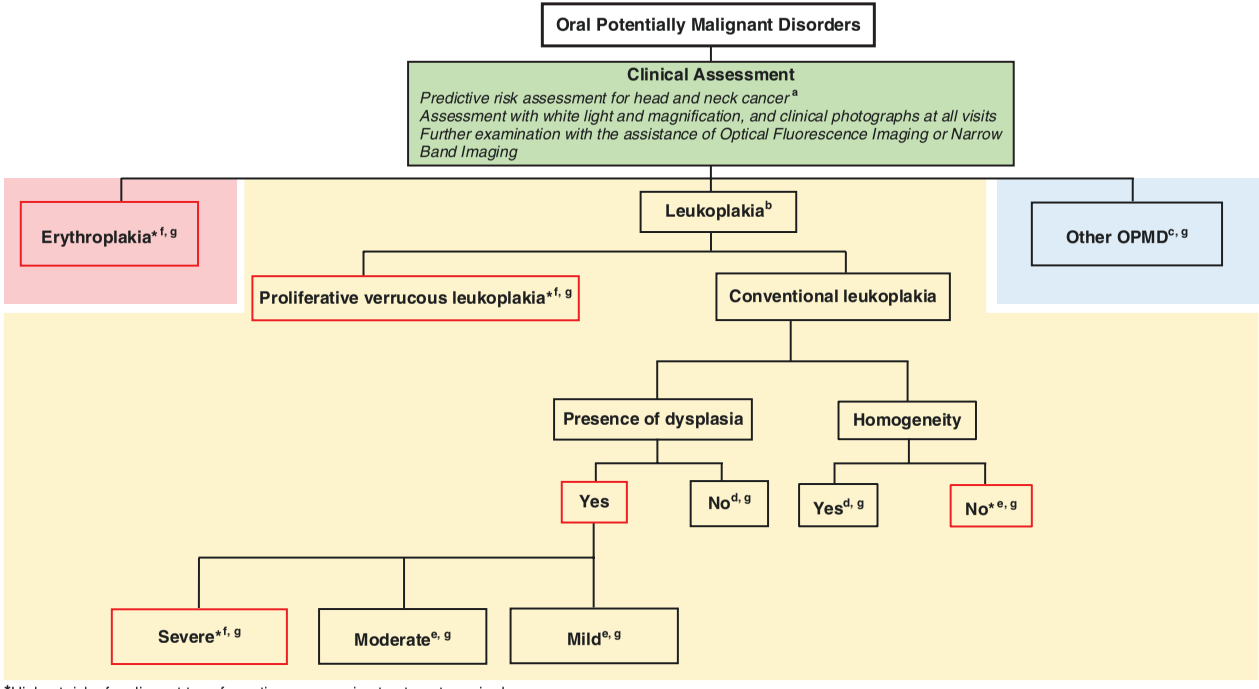
In summary, the philosophy of OPMD management is centred around the prevention of oral squamous cell carcinoma development and on the preservation of life quality. Identification of disease progression and facilitation of the follow-up process constitute important treatment benefits. Ultimately, various disease and patient characteristics impact on the decision to initiate treatment or recommend surveillance.

Treatment Recommendations for Oral Potentially Malignant Disorders

The answer to the question when to treat OPMDs is complicated by the current lack of clear evidence-based guidelines regarding the optimal management of these disorders. With this in mind, at present, our treatment recommendations are summarised in Fig. 11.2. Several factors require consideration, and these may vary depending on the particular OPMD in question, the affected individual's comorbidities and their wishes. These factors are discussed below.

Presence of Symptoms and/or Disability

The need for symptom relief and the restoration of compromised oral function may, in some cases, be the motivating factor instigating treatment. While all OPMDs have the potential to create pain or discomfort, this is particularly evident in OLP, DLE and cGVHD, where the main goal of treatment is symptom control [41, 47].



*Highest risk of malignant transformation –aggressive treatment required.
a See document entitled 'Predictive risk assessment for head and neck cancer'.
b See document entitled 'Predictive risk assessment for malignant transformation of oral leukoplakia'.
c Manage accordingly if disabling, symptomatic or ulcerated; Elimination of modifiable risk factors for oral SCC; Indefinite follow-up every 6-12 months; Re-biopsy treatment non-responsive sites.
d Complete excision if <2-3cm; Elimination of modifiable risk factors for OSCC; Indefinite follow-up of treated and untreated patients every 6 months; Periodic biopsies if complete excision not possible.
e Complete excision should be undertaken if acceptable post-operative function is achievable; Elimination of modifiable risk factors for OSCC; Indefinite follow-up of treated and untreated patients every 3-6 months; Periodic biopsies if complete excision not possible.
f Complete excision with reconstruction as required; Elimination of modifiable risk factors for OSCC; Indefinite follow-up every 3 months.
g Biopsy or excision is undertaken with the assistance of Optical Fluorescence Imaging or Narrow Band Imaging to determine the true extent of the lesion and clear surgical margins.

Fig. 11.2 Management guidelines for oral potentially malignant disorders (Image adapted and used with permission from C. S. Farah et al. (eds.), Contemporary Oral Medicine, Springer Nature Switzerland AG 2019)

Oral submucous fibrosis is not only potentially associated with significant oral burning and pain but is also a progressively disabling disorder, and treatment is additionally aimed at halting disease progression and improving the disability [23, 48]. It has been suggested that the reduction of excessive fibrosis by appropriate treatment may also have the added advantage of decreasing the risk of development of OSCC by reducing tissue hypoxia and the subsequent expression of genes, such as VEGF, which are known to play a role in carcinogenesis [26].

Assessment of Risk Factors

Numerous risk factors have been identified which increase the probability of malignant transformation of OPMDs. Careful consideration of these factors impacts on the decision to initiate treatment or recommend surveillance. Oral epithelial dysplasia is regarded as the single most important predictive factor [27]. Several other risk factors have also been described particularly with reference to oral leukoplakia—the most common OPMD [44].

Risk factors are easily divided into patient risk factors and lesion risk factors. Lesion risk factors are related to the clinical appearance of the lesion and should be assessed with careful clinical examination under good illumination preferably with white light and magnification. Further examination with the assistance of adjunctive optical devices such as optical fluorescence imaging or narrowband imaging may be helpful (Fig. 11.3). Lesion risk factors of note include clinical appearance, site, size, multifocality and duration of lesion. Patient risk factors include age, gender, smoking habit, alcohol exposure and family history. Patient and lesion risk factors are summarised in Fig. 11.4 and detailed below.

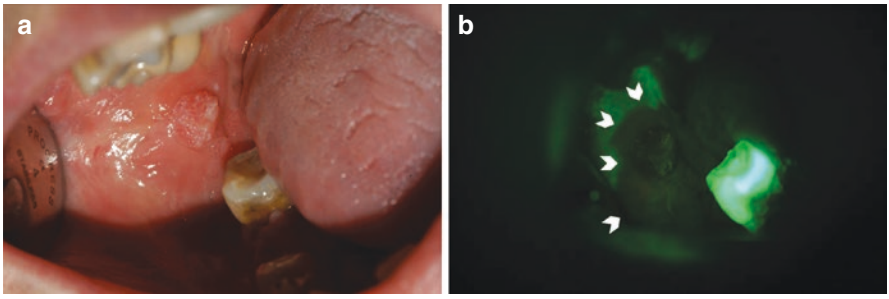


Fig. 11.3 Papillary lesion on the right buccal mucosa under white light (**a**). Papillary lesion on the right buccal mucosa with optical fluorescence imaging; VELscope Vx® (**b**) which enhances visualisation of the lesion and determines clearer delineation of margin (arrowheads). Lesion was biopsy-proven papillary squamous cell carcinoma

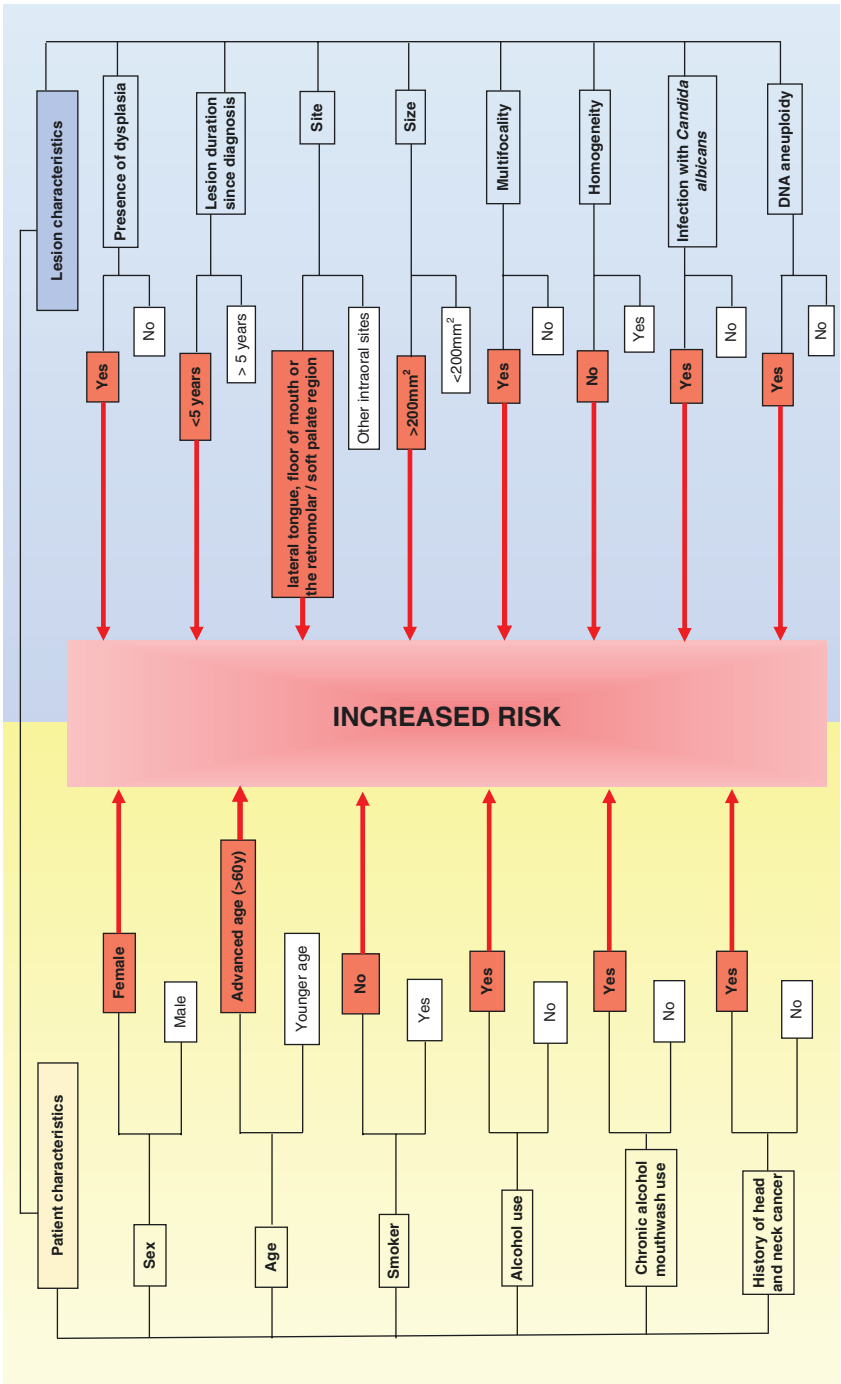


Fig. 11.4 Predictive risk factors for malignant transformation of oral leukoplakia (Image adapted and used with permission from C. S. Farah et al. (eds.), Contemporary Oral Medicine, Springer Nature Switzerland AG 2019)

Patient Risk Factors

Age

Evidence from observational studies identifies advanced age as a significant determinant of the malignant potential of leukoplakia [44]. Advanced age however may potentially limit treatment options given the greater risk of other associated comorbidities. In a Swedish study, malignant transformation was most common in 782 patients with leukoplakia in the 70–89 age group (7.5%) compared with 1% in patients under 50 years [49]. In a Hungarian study, the peak incidence of leukoplakia was in the sixth decade, whereas the peak incidence of carcinoma arising in leukoplakias was in the seventh decade [50]. In an Indian study, the highest prevalence of leukoplakia occurred in the 35–54-year age group; however malignant transformation of pre-existing leukoplakia occurred in the 55–74-year age group [51].

Gender

While in general, the majority of leukoplakias occur in middle-aged and elderly males, the female gender has been associated with increased risk of malignant transformation [12, 44]. Studies investigating malignant transformation of OPMD have found females to be at increased risk compared to males. These include studies from Sweden [49], Denmark [52], Norway [53], the Netherlands [54], the USA [55] and Hungary [50]. In contrast, the rare proliferative verrucous leukoplakia (PVL), a distinct and most lethal form of the disease, exhibits a very strong female predilection, with about 67% of cases occurring in this population group [56]. This increased tendency of leukoplakias in females to become malignant should be considered when treatment decisions are made. Older females with OLP involving at-risk sites appear to be at higher risk [57], but general agreement among researchers is lacking [58].

Smoking Status

Tobacco use is the most common predisposing factor for the development of an intraoral white lesion, although a certain proportion of oral white patches have no known cause. In a number of regions, tobacco use and areca nut/betel leaf use, either alone or in combination (betel quid), account for the vast majority of leukoplakias. In Gujarat, only 15% of 57,518 mill workers aged over 35 years did not habitually use tobacco or areca nut, and only 2% of those with a mucosal lesion did not have a tobacco-related habit [59]. Over a 2-year period, all patients who developed squamous cell carcinoma had a smoking habit. In the Western world, most leukoplakias are detected in smokers [60–62]. The method of tobacco use influences the clinical distribution of lesions, e.g. ‘reverse’ cigarette smoking causes

lesions on the hard palate, chewing causes lesions at the site of quid placement, and smoking of cheroots is associated with floor of mouth leukoplakias [63]. A recent study of clinical features of OPMD in an Australian population showed 63% of patients with dysplastic lesions were smokers [45].

Leukoplakia in non-smokers carries an increased risk of malignant transformation. In a Swedish study, the 5-year cumulative risk for malignant transformation was 3.1% in non-smokers compared with 0.4% in smokers [49], and a similar trend was found in a Danish study [52]. In Hungarian subjects, there is a greater propensity for malignant transformation in leukoplakias in nontobacco users [50, 64]. In Californian patients, malignant transformation occurred in a higher proportion of non-smokers, but in smokers malignant transformation occurred in a higher proportion of those who continued to smoke after leukoplakia was detected compared with those who ceased [55]. Schepman et al. found non-smoking female patients with leukoplakia to be at a statistically significant risk of malignant transformation compared with women who smoked, while no such relationship could be determined for men [54].

Overall, the risk of malignant transformation of OPMD in non-smokers has been shown to be higher compared to smokers [54, 65]. Dysplastic lesions in non-smokers are seven times more likely to transform [66]. Following removal however, smokers have been shown to be at a significantly higher risk of recurrence of dysplastic lesions, compared to ex-smokers or non-smokers [67]. Smoking status may therefore impact on the decision to surgically treat extensive lesions, where a functional compromise is likely; surgical risks need consideration, and the risk of recurrence is significant. Irrespective of the presence or absence of an OPMD, tobacco smoking is considered a major risk factor for oral SCC, and smoking cessation should form an integral part of the treatment programme of any affected individual with an OPMD [68].

The high-risk nature of lesions in non-smokers is of particular importance and requires a greater emphasis and recognition among clinicians dealing with OED, as it suggests that those non-smokers with OED have an inherited or acquired predisposition and should be treated more aggressively [66]. Smoking and alcohol use do not appear to be risk factors for the development of OSCC in patients with OLP [69].

Alcohol Exposure

Alcohol consumption is an independent risk factor for oral cancer [70]. The use of alcohol in combination with tobacco appears to have a greater than multiplicative risk effect for OSCC [68]. Consumption of as little as 12 g of ethanol per day has been shown to increase the risk of oral and pharyngeal cancer, and the danger rises with increasing consumption [71]. The role of alcohol as an independent factor for oral leukoplakia development is not as well documented as tobacco with one study finding that alcohol doubled the risk of malignant transformation [72]. Not surprisingly, it has been shown that alcohol use increases the risk of malignant

transformation of OPMDs, and its regular consumption has been linked with increased risk of dysplasia [45]. It is also important to note, as is the case with tobacco use, that excessive alcohol consumption is a key risk factor for chronic disease, leading to poor general health and disability, potentially limiting the available treatment options in patients with OPMDs [73]. All individuals with OPMDs should therefore be counselled about the importance of limiting their alcohol exposure.

Increased risk of oral cancer has also been attributed to the regular use of alcohol-containing mouthwashes [74, 75], although a meta-analysis published by Gandini et al. [76] failed to demonstrate a significant association. It is however important to make the point that the current absence of evidence does not necessarily translate to a definitive evidence of no risk. The link between alcohol-containing mouthwash use and increased risk of oral cancer is a plausible one, and a unifying hypothesis has recently been published by Currie and Farah [75]. Topical exposure to alcohol-containing mouthwashes can contribute to carcinogenesis by significantly increasing the level of salivary acetaldehyde [75]. Ethanol is also known to enhance the actions of tobacco-related carcinogens by inducing cytochrome P450 2E1 and increasing the penetration of the oral mucosa [75]. The use of alcohol-containing mouthwashes by patients who already present with oral epithelial dysplasia is particularly concerning as this practice may facilitate malignant transformation of these lesions [75]. At present, the link between alcohol-containing mouthwashes and oral cancer remains an area of controversy, and it is yet to be established whether a definitive cancer risk exists and, if so, if the risk is the result of the ethanol content of the product, is related to the underlying oral health conditions necessitating the use of the mouthwash in the first instance or both [77].

Personal and Family History of Cancer

History of previous head and neck cancer has been reported as a significant risk factor for malignant transformation of oral leukoplakia [43]. A large population-based case-control study in France reported a higher risk of oral cavity cancer among individuals with first-degree relatives with head and neck cancer history, compared to subjects without a family history. The risk increased with the number of affected relatives [78]. The study did not find a significant relationship between the risk of oral cavity cancer and family history of non-head and neck cancers. This finding is similar to previous studies [79–82]. An increased risk of OSCC associated with a family history of head and neck cancer has been observed in non-smokers and consumers of low quantities of alcohol, but the risk increased with the exposure [78]. These findings are also similar to those of earlier studies [79, 81]. A recent study has also shown that women with cervical cancer were almost seven times more likely to develop head and neck cancer compared to the general female population [83].

Rare conditions including Fanconi's anaemia, Bloom's syndrome, xeroderma pigmentosum and Li-Fraumeni syndrome are associated with increased risk of oral cancer in the absence of other risk factors [84]. Relatives of patients with oral squamous cell carcinoma may be at increased risk [82, 85].

There has been less work exploring the genetic susceptibility for OPMD compared to OSCC. Based on significant associations as reported by two or more studies, Shridhar and colleagues [86] have recently summarised suggestive markers which included single nucleotide polymorphisms (SNPs) in *GSTM1* (*null*), *CCND1* (*G870A*), *MMP3* (*-1171; promotor region*), *TNFa* (*-308; rs800629*), *XPD* (*codon 751*) and *Gemin3* (*rs197412*) as well as in *p53* (*codon 72*) in Indian populations. However, an equal or greater number of studies reported null or mixed associations for SNPs in *GSTM1* (*null*), *p53* (*codon 72*), *XPD* (*codon 751*), *XRCC* (*rs25487 C/T*), *GSTT1* (*null*) and *CYP1A1m1* (*MspI site*) [86].

Furthermore, hyper-methylated loci reported in three or more studies included *p16*, *p14*, *MGMT* and *DAPK*. Two longitudinal studies reported greater *p16* hypermethylation in precancerous lesions transformed to malignancy compared to lesions that regressed (57–63.6% versus 8–32.1%; $p < 0.01$). The one study that explored epigenome-wide methylation patterns reported three novel hyper-methylated loci (*TRHDE*, *ZNF454*, *KCNAB3*) [87]. Clearly more work is required to explore the genetic susceptibility of OPMD generally and oral leukoplakia specifically.

Environmental Exposure

Oral and oropharyngeal cancer risk is 87% higher in never-smokers who have ever been exposed to environmental tobacco smoke at home or work, compared with unexposed never-smokers [88]. Exposure to asbestos or to polycyclic aromatic hydrocarbons has been linked with oral and pharyngeal cancer risk [89] as has exposure to solvents and possibly pesticides, engine exhaust, textile dust and leather dust [90]. Occupations related to the pulp industry, wood or wood products or exposure to chemicals, e.g. phenoxyacetic acid, has been implicated as risk factors for oral cancer [91]. Other studies have found a significantly lower risk of oral and pharyngeal cancer in wood workers [90].

In a Taiwanese study [92], patients with OPMD who resided in areas with high nickel concentrations (polluted levels) exhibited hazard ratios of 1.8–2 for oral cancer relative to those who lived in areas with low nickel levels ($p < 0.01$). Meanwhile, smokers with OPMDs had a hazard ratio of 2.8–2.9 relative to non-smokers. Betelquid chewers had a 2.2–2.3 hazard ratio relative to non-chewers. Smoking, betelquid chewing and environmental nickel exposure were associated with an increased risk of oral cancer development in patients with OPMD [92].

Actinic cheilitis (AC) is a potentially malignant disorder of the lip caused by chronic exposure to solar radiation. The prevalence of AC varies based on geographical location with general population studies in Europe reporting prevalence of 0.8% in patients with oral mucosal disease [93] while studies from Brazil reporting prevalence as high as 39.6% among agricultural workers [94]. AC is common in patients of fair skin who spend significant amounts of time outdoors exposed to sun exposure with minimal protection, including farmers [95] and beach workers [96].

Lesion Risk Factors

Site

The site of OPMD impacts on the risk of progression to malignancy and should be factored into any treatment decision. While most leukoplakias occur on the buccal mucosa, those presenting on the lateral border of the tongue and in the floor of the mouth or the retromolar/soft palate region are associated with the highest risk of malignant transformation [12, 45]. The lateral border of the tongue has also been correlated with a higher risk of malignant transformation of dysplastic lesions compared to those affecting other intraoral subsites [66].

The floor of the mouth and lateral border of the tongue, sites where OSCC are frequently seen, seem disproportionately associated with the subsequent development of cancer. These two high-risk sites for malignant transformation are supported by studies from Denmark [52], Hungary [64], England [97] and the USA [55]. Such an association has not been found in all studies including that by Schepman [54]. In that study, 15 patients from 101 with lesions on the tongue or floor of the mouth developed OSCC compared with five from 65 whose lesions were located elsewhere, although this did not reach statistical significance [54]. In the study by Holmstrup et al., in which most patients with OPMD at so-called 'high-risk' sites such as the tongue and floor of the mouth underwent surgical excision, an equal proportion of lesions at other sites developed OSCC [98]. Numerous studies have reported the incidence of malignant transformation of OLP lesions to be greater for tongue lesions compared with buccal mucosal lesions, and malignant lesions have been reported to occur in plaque-like OLP on the dorsum of the tongue, an unusual site for OSCC [99].

Size

The risk of progression to malignancy has been reported to be significantly higher in leukoplakias larger than 200 mm^2 [44]. The same holds true for dysplastic lesions, with lesions greater than 200 mm^2 having been shown to be three times more likely to undergo malignant transformation compared to smaller lesions [66]. Large lesions, while carrying a higher risk of malignant transformation, are unfortunately also more difficult to manage as complete removal may not leave the individual with an acceptable functional compromise. Furthermore, other comorbidities become significant, impacting the surgical risk.

Multifocality

Widespread, multiple oral leukoplakias appear to exhibit a higher potential for the development of squamous cell carcinoma than do localised lesions [100]. Higher risk of malignant transformation has also been described for multiple oral dysplastic

lesions compared with single lesions [101]. Proliferative verrucous leukoplakia, with its high risk of progression to malignancy, is also characterised by its multifocal nature [56]. As is the case with large lesions, multifocal lesions are more difficult to manage surgically, and, in the case of extensive lesions, close follow-up with regular biopsies may constitute the necessary compromise.

Duration

Oral potentially malignant disorders are chronic conditions with an increased risk of malignant transformation, particularly in the first 5 years after diagnosis [12]. While the rate of malignant transformation may decrease thereafter, the risk does not completely disappear [12]. The longer the follow-up period, the higher the number of transformed lesions. Some leukoplakias have been shown to transform up to 16 years of follow-up [53]. In light of this, lifelong surveillance of both treated and untreated patients with oral leukoplakia has been suggested [43].

Clinical Appearance

Leukoplakia is considered to be a potentially malignant lesion, but the term 'disorder' rather than 'lesion' is more appropriate, recognising the fact that malignant transformation does not always take place in the leukoplakic area but may occur elsewhere in the mouth or the upper aerodigestive tract [102].

Oral leukoplakia can show a variety of clinical appearances. Some are uniformly white and flat (termed 'homogenous leukoplakia'), while others are non-homogenous with a warty or nodular appearance, perhaps predominantly white with red areas or largely red with white speckles ('erythroleukoplakia'). Lesions containing nodular or red areas have been shown to carry a greater risk of malignant transformation [52]. Homogenous lesions carry a lower risk of malignant transformation [50, 97]. Lesions with red areas have a higher incidence of malignant transformation [53, 55]. In the Netherlands, a statistical association was found between non-homogenous OPMD and the development of OSCC with a sevenfold increased risk when compared with homogenous lesions [98]. The size of lesions has been investigated in some studies with larger lesions more at risk of malignant transformation [52, 98].

Although the most common type of oral leukoplakia is the homogenous type, it is those lesions which contain nodular and/or red areas (the non-homogenous type) which are associated with a four to seven times greater risk of malignant transformation [40, 44, 45]. PVL represents the most recalcitrant and concerning variant of oral leukoplakia presenting initially as flat homogenously white patches which tend to reoccur and proliferate with time to become multifocal [56]. A recent systematic review identified a 63.9% malignant transformation rate of PVL over a mean follow-up period of 7.4 years [56]. Early diagnosis, aggressive intervention and close surveillance with regular biopsies are critical to the successful management of this disorder. Unfortunately, this is often complicated by the retrospective nature of the diagnosis in many instances.

Actinic cheilitis is mostly found on the lower lip and can present as a hyperkeratotic lesion, although dryness, atrophy, scaliness, blotchiness, erythema, ulceration and loss of vermillion border distinctness. Homogenous and non-homogenous plaques are often seen, but these tend to be late presentations [103].

Presence of Dysplasia

The presence of epithelial dysplasia has been associated with malignant transformation particularly for lesions involving the tongue or floor of the mouth. Assessment of dysplasia is subjective with both inter- and intra-observer variation [104, 105]. The malignant transformation rate of dysplasia has been reported to range from 4.7% to 36.4% [16, 46], with the risk increasing with the degree of dysplasia [16, 46].

A recent study found the overall transformation rate of OPMD was 4.32% [106]. Additionally, the mean time of malignant transformation was significantly shorter for lesions with than without epithelial dysplasia [106]. The risk of malignant transformation was 1.89 times higher for dysplastic than non-dysplastic lesions. The anatomical site of OPMD and the presence of epithelial dysplasia were significantly associated with malignant transformation [106]. In an Australian study, 368 patients with a histologically confirmed diagnosis of epithelial dysplasia were identified [46]. Twenty-six (7.1%) underwent progression or malignant transformation, of which 18 (4.7%) developed OSCC, in a mean time of 3.3 years. The highest rates of progression or malignant transformation were among females and patients over 45 years of age. For all oral sites and dysplasia grades, the annual malignant transformation rate was approximately 1%, and the highest transformation rate by site was the tongue (1.4%). Regarding the grade of dysplasia, 4.1% mild, 7.1% moderate and 1.8% severe dysplasias underwent malignant transformation. The severity of epithelial dysplasia was not associated with an increased risk of progression or malignant transformation suggesting that the current grading system of oral epithelial dysplasia is not useful for predicting patient outcomes or for determining management strategies [46]. As stated above, non-smokers with OED may have an inherited or acquired predisposition and should be treated more aggressively [66].

Terminology based on histopathological criteria potentially leads to confusion with the concept of lichenoid dysplasia introduced by Krutchkoff and Eisenberg to describe lesions with features of both OLP and epithelial dysplasia [107]. Van der Meij proposed the term oral lichenoid lesion (OLL) for cases defined as clinically typical but histologically compatible or clinically compatible and histologically typical or both clinically and histologically compatible with OLP [108], and several authors have since proposed that OLL rather than OLP is at risk of malignant transformation [58, 109, 110].

Although at present no consensus exists regarding the ideal management protocol of dysplastic lesions, current evidence suggests that surgical excision decreases

the risk of progression to malignancy, when compared to observation alone [111–113]. The recently published management logarithm for dysplastic lesions by Field et al. [16] recommends surgical excision of all resectable lesions with severe dysplasia or carcinoma in situ, with surgical reconstruction, as required. Complete excision is also recommended for moderately dysplastic lesions if acceptable postoperative function can be achieved. The later recommendation similarly applies to mildly dysplastic lesions when other predictive risk factors [66] for malignant transformation are also present, namely, non-smoking status, site (lateral border of tongue), non-homogenous appearance and lesion size in excess of 200 mm^2. Farah and colleagues posit that definitive treatment of all dysplastic lesions, irrespective of the degree of dysplasia, is supported by the poor predictive value of dysplasia grading [46]. Given that the severity of dysplasia has not been demonstrated to consistently correlate with the risk of malignant transformation, this calls into question the ethics of the 'wait and watch' approach often applied to the mildly dysplastic cases [46].

DNA Aneuploidy

The presence of DNA aneuploidy (abnormal content of nuclear DNA) in oral leukoplakia has been correlated with an increased risk of malignant transformation [114–116]. Abnormal DNA content in oral epithelial dysplasia has also been associated with an increased risk of progression to carcinoma [117]. In a recently published large series of 1401 individuals with OPMDs, DNA ploidy was shown to be generally associated with the grade of epithelial dysplasia and to have a high predictive value for malignant transformation of the disorder [118].

Presence of Invasive Candida

Candida albicans biotypes are capable of producing the carcinogen nitrosamine *N*-nitroso-benzylmethylamine, which may play a role in the causation of OSCC [119]. More recently, it has also been shown that *Candida albicans* are capable of metabolising ethanol to acetaldehyde, another known carcinogen, and that this phenomenon appears enhanced in smokers [120]. The presence of *Candida albicans* is often mentioned as a risk factor for malignant transformation of oral leukoplakia [15]. Wu et al. in a study examining 396 leukoplakias found the frequency of *Candida* infection to be 15.9%. Patients older than 60 years, lesions located on the tongue and the presence of dysplasia were significant risk factors for *Candida* infection in oral leukoplakia [121]. Chiu Chang-Ta et al. in a retrospective study conducted on 136 smokers with oral leukoplakia found *Candida* to be an important risk factor in patients who smoke with multiple oral leukoplakias, and multiple oral leukoplakias with *Candida* infection were more likely to exhibit dysplasia [122].

Control of Inflammation

Given that a link has been established between chronic inflammation and cancer [123], it stands to reason that treating chronic oral mucosal inflammatory disorders is important, although the inflammatory process on its own may not necessarily be the sole factor responsible for the carcinogenesis [124]. Several mechanisms have been proposed. Chronic inflammatory mediators have the capacity to induce cell proliferation and prolong cell survival by activating oncogenes and inactivating tumour suppressor genes [125, 126]. Inflammatory cells may also secrete reactive oxygen and nitrogen species, which have the capacity to damage DNA directly and dysregulate the mechanisms of DNA repair and apoptosis, creating genomic instability [125]. Chronic inflammation, if persistent, therefore has the potential to create an environment in which a cancer is more likely to develop.

Oral lichen planus and cGVHD-related inflammatory reactions have been proposed as contributory mechanisms leading to the increased risk of malignant transformation seen in these disorders [41, 47]. Treatment of OLP with topical corticosteroids has been shown to significantly decrease levels of pro-inflammatory cytokines involved in the development of the OSCC such as TNFα, IL-1α, IL-6 and IL-8 [25]. Considering that the cytokine microenvironment associated with OLP can promote tumour progression, it has been suggested that by eliminating the inflammatory response through treatment and by the restoration of the normal immune response, it may be possible to interrupt the progression to cancer [41]. Interestingly, in an Italian study of 402 individuals with OLP, followed up for an average period of 4.9 years, where the majority of participants were treated with topical and/or systemic corticosteroids, it was observed that immunosuppressive therapy did not influence the risk of malignant transformation [127].

Transforming growth factor beta (TGF-β) is one of the key molecules associated with the initiation of fibrosis in OSMF. This multifunctional cytokine has a range of biological effects and is believed to play a role in malignant transformation not only in OSMF [26] but also potentially in OLP [126]. It has been suggested that treatment with TGF-β inhibitors may decrease inflammation, fibrosis and the malignant potential of OSMF [48]. Inflammatory molecules such as IL-6, IL-8 and GRO-α have also been suggested to play a role in the malignant transformation in OSMF [26].

Persistence of the Disorder

In some situations, as may be the case with oral leukoplakia, where resolution of the disorder is possible, it is not unreasonable to allow a period of surveillance in which to observe for possible regression of the disorder. Resolution rates of up to 42.5% of untreated leukoplakias have been demonstrated in some population studies [28]. Considering the invasive nature of the currently recommended surgical intervention

[43], if the likely aetiological factors such as tobacco use or infection with *Candida albicans* can be eliminated, an observation period of up to 6 weeks is generally acceptable during which to monitor the lesion for signs of regression, acknowledging that complete resolution may take longer [27, 43]. This approach however is only feasible if the elimination of the identified risk factors is realistically achievable. If signs of regression are not observed, the disorder should be treated, if possible.

Amenability to Treatment

Oral epithelial dysplasia is considered an important prognosticator of future malignant transformation in an OPMD, and evidence exists to support its complete excision [46, 113]. The European Academy of Oral Medicine diagnostic and therapeutic protocols for oral leukoplakia and erythroplakia currently also recommend that all leukoplakias and erythroplakias be surgically treated, if such treatment is possible [43]. Surgical intervention however may not always be feasible. This may be the case with large or diffuse lesions, if the affected individual's comorbidities place them at an unacceptable level of surgical risk, or if the affected individual does not consent to treatment [15].

Observation of Change

Observation of change either in the appearance of the lesion or its behaviour may signal progression to malignancy. This change may take the form of mucosal thickening, appearance of erosion/ulceration or emergence of pain. In the case of OPMDs such as OLP, DLE or cGVHD, the lack of response to treatment of localised erosive or ulcerated lesions should raise suspicion of possible progression. Diagnosis of PVL is particularly problematic, as it is often made retrospectively. In the early stages, proliferative verrucous leukoplakia may be indistinguishable from a solitary, conventional homogenous leukoplakia [56], and it is not until a change in appearance and behaviour is observed over time that a correct diagnosis can be made, highlighting the importance of long-term follow-up. In some instances, it may be that observation of change either in lesion appearance or its behaviour may constitute the motivating factor to treat a particular OPMD, which may otherwise have simply been observed. It has been argued that definitive treatment of oral leukoplakia with dysplasia, or leukoplakia with high-risk features, is the best first step for the observation of change [45, 46] as any changes that do appear are likely to be more noticeable on a background of normal-looking mucosa compared to mucosa affected by leukoplakia and/or erythroplakia [40].

For actinic cheilitis, the overall philosophy for treatment includes a thorough assessment of the patient to ensure that any required short-term treatment is followed

by a reasonable medium- to long-term management plan [103]. Dysplasia at some level is an intrinsic component of most cases of actinic cheilitis and is an unpredictable tissue change in the progression from normal epithelium to malignancy. While malignant transformation is not inevitable, it is an important consideration in treatment planning and observation. If treatment is not to be instigated early after the clinical diagnosis of actinic cheilitis, then close observation in addition to adherence to preventative measures is paramount. The lip is quite amenable to observation, but the long lag time between onset of actinic cheilitis and change to malignancy places more importance on accurate recording of changes and close surveillance strategies.

Treatment Modalities for Oral Potentially Malignant Lesions

There is no convincing evidence that treatment of OPMD prevents progression to OSCC [128]. The prevention of malignant transformation of OPMDs is the primary aim of treatment, and as yet there is no consensus on the best modality of treatment to achieve this [98].

The contemporary clinician is compelled to treat patients in line with evidence-based practice. The challenge for the clinician treating OPMDs is that overall the literature is low in hierarchy of evidence, is heterogenous and spans decades over which there have been significant changes in diagnosis and management. Most studies have methodological and reporting flaws, making it challenging to discern meaning from them [129].

The majority of the literature about the treatment of OPMDs pertains to the treatment of oral leukoplakia, the most common OPMD, but several studies also report on treatment of actinic cheilitis. Treatment modalities and the evidence for the efficacy of topical medical treatments, systemic medical treatments, surgical treatments and photodynamic therapy will be discussed below for various conditions.

Medical Treatment

There are no medical treatments that are effective in treating oral potentially malignant disorders to prevent malignant transformation [128–130].

Topical Medical Treatment

Vitamin A/Retinoids

Vitamin A compounds are required as part of normal cell growth. Retinoids (vitamin A derivatives) have been shown to affect gene expression [131] and suppress carcinogenesis [132, 133]. Vitamin A is also an antioxidant [134]. Topical retinoids

have been proposed as treatment for OPMDs, particularly oral lichen planus and oral leukoplakia.

Only a single, very small, randomised controlled trial has compared topical retinoid therapy to placebo. In 1999, Paitelli et al. compared topical 13-cis-retinoic acid to placebo. The topical retinoic acid showed no difference in resolving oral leukoplakia compared to placebo [135].

Multiple, small, prospective studies have failed to demonstrate that topical application of retinoids can treat oral potentially malignant disorders. Across these studies, most patients with a complete response experienced recurrence when treatment was withdrawn [136–138].

Topical vitamin A therapies have not been demonstrated to effectively treat OPMDs, and there is no evidence they prevent progression of these lesions to oral malignancy.

Bleomycin

Bleomycin is a chemotherapeutic agent that acts by inducing breaks in DNA strands [131]. In the 1990s, bleomycin was investigated for potential use as treatment for oral leukoplakia in a randomised, double-blind trial [139]. This small trial failed to demonstrate a difference in cancer development between the test and placebo groups. A separate prospective study again failed to demonstrate prevention of malignant progression with topical bleomycin [140], and this treatment approach has been abandoned.

Anti-inflammatory Agents

It has been demonstrated that there is a marked increase in cyclooxygenase-2 expression in HNSCC, as well as in the ‘normal’ tissue adjacent to the margins, when compared to oral mucosa controls [141]. Renkonen et al. demonstrated that the level of COX-2 expression in squamous cell carcinoma of the tongue increased in a gradient fashion from normal oral mucosa to dysplastic mucosa to carcinoma [142].

Investigators have assessed whether blocking this pathway with a cyclooxygenase inhibitor may decrease the rate of progression of OPMD. In the mid-2000s, a randomised, placebo-controlled, double-blind trial compared an oral rinse containing ketorolac (an anti-inflammatory agent) with a placebo oral rinse [143]. There was no difference in progression of OPMD between the groups nor any difference in response rate (as measured by oral examination and colour photographs). A case series of six patients with actinic cheilitis treated with a 6-week course of topical 3% diclofenac demonstrated clinical and histopathological resolution of actinic cheilitis at the conclusion of therapy in only four patients, with no follow-up data supplied [144].

Antimycotic Agents

The association between oral leukoplakia and *Candida* infection date back half a century [145]. *Candida* infection is proposed to play a role in the carcinogenesis of oral potentially malignant disorders [146]. The rate of infection with *Candida albicans* in the presence of OPMD has been reported with a wide variation, with studies quoting between 15% [147] and 60% [148].

The European Association of Oral Medicine protocol for the management of leukoplakia and erythroplakia suggests treating oral leukoplakia with topical medication to eradicate *Candida* for a maximum of 6 weeks [43]. Successful treatment of oral *Candida* and oral leukoplakia with antifungals has been described for many years, since the early link between *Candida* infection and oral malignancy was described [149]. While it is commonly held that topical antifungals may improve the appearance and symptoms of chronic hyperplastic candidosis, there is no evidence that topical antifungals inhibit the progression of oral potentially malignant disorders to oral malignancy [129].

Gene Therapy

Gene therapy is a relatively new concept. Gene therapy has been defined as 'the in vivo or ex vivo introduction of nucleic acids that regulate gene expression or convert prodrugs into cytotoxic agents in target tissues, resulting in a therapeutic benefit' [150].

It is known that around half of all head and neck squamous cell carcinomas (HNSCC) harbour a p53 mutation [151]. Alterations in p53 have been demonstrated to occur early in the tumourigenesis of HNSCC—and have been demonstrated in oral potentially malignant disorders [152]. Topical gene therapy for oral potentially malignant disorders has been developed to take advantage of these defects in p53-related pathways [153].

A mouthwash termed 'ONYX-015' selectively kills cells with defects in this intracellular signalling pathway [153]. A feasibility study examining the role of ONYX-15 for treatment of oral potentially malignant disorders published in 2003 reported resolution of dysplasia on histological analysis in 37% of the small sample size of 19 patients [153]. This effect was transient, and this has not been shown to be efficacious in a randomised controlled trial [128].

5-Fluorouracil

5-Fluorouracil is an antimetabolite chemotherapeutic agent which has been used in the treatment of actinic cheilitis [154]. Anecdotally, significant local side effects are reported to account for poor patient compliance with this therapeutic option [154]. In a randomised trial of actinic cheilitis treatments performed in 1989, ten

patients were treated with 5% topical fluorouracil for 2 weeks [155]. Biopsy at 1 year demonstrated treatment failure in more than half of the patients, with persistent cellular atypia and dysplasia. In 1981, Warnock et al. reported a histopathological treatment failure rate of 100% in six patients treated with 5-fluorouracil for AC [156].

Chemical Peel

Topical trichloroacetic acid did not successfully treat actinic cheilitis in any of the ten patients treated in a randomised trial [155]. It is an ineffective treatment method [154] and should not be used in the treatment of actinic cheilitis.

Imiquimod

The first data on the use of imiquimod for actinic cheilitis was published in 2002: a small series of 15 patients with biopsy-proven actinic cheilitis, treated with 4–6 weeks of topical imiquimod [157]. Only 60% reached the short 3-month follow-up period, and all had clinical resolution of lesions [157].

Ingenol Mebutate

A recent paper reports outcomes for a small series of seven patients with actinic cheilitis treated with ingenol mebutate gel. Post-treatment histopathology was not performed, and complete clinical response was reported in less than half of the patients [158]. An earlier case series of four patients treated with ingenol mebutate reported clinical response in only two patients.

Systemic Medical Treatment

Steroids

Systemic steroids have been used by some practitioners for the treatment of oral potentially malignant disorders. In 2007, Epstein et al. surveyed 176 oral medicine professionals regarding their management of oral potentially malignant disorders [159]. Around 40% of responders stated that they would use systemic steroids for the treatment of mixed-striated lesions, and 26% recommended systemic steroids for both clinically detected red (oral erythroplakia) and white lesions (oral leukoplakia).

There is no evidence that systemic steroids prevent malignant progression of oral potentially malignant disorders.

Retinoids

Retinoids are a group of compounds that have similar activity to vitamin A [130]. Retinoids exert their effect on cell development by altering gene expression [132]. Treatment of OPMDs with retinoid supplementation was first investigated in the 1960s [130]. To date, there is no evidence that systemic vitamin A (a retinoid) alters carcinogenesis in oral potentially malignant disorders.

Three randomised controlled trials compared systemic vitamin A or other retinoids to placebo. Two of the studies showed some benefit. Both studies were very small, with 21 and 42 patients taking vitamin A in each study, respectively [160, 161]. In one of the studies [161], 64% of complete responders had recurrence of leukoplakia after ceasing supplementation.

The third of the randomised controlled trials compared 13-cis-retinoic acid to placebo [162]. Again the sample size was very small, with only 24 patients taking the active treatment. No difference in resolution of the histologically confirmed oral potentially malignant lesions (measured by gross inspection) was noted between the two groups. Although a difference was demonstrated between the two groups when partial and complete responses were reviewed together, the majority of those who responded to retinoid therapy relapsed, typically 2–3 months after stopping treatment. Seventy-nine percentage of patients taking retinoids experienced side effects, which included conjunctivitis and hypertriglyceridemia in over 50% of patients.

Systemic retinoid treatment has not been shown to prevent carcinogenesis in OPMDs. Prospective trials demonstrating clinical resolution with retinoid therapy in some cases of OPMD have been accompanied by high levels of recurrence on withdrawal of therapy and not insignificant side effects [163]. Systemic treatment with vitamin A-related compounds is not appropriate in the management of OPMDs.

Beta Carotene or Carotenoids

Carotenoids act as antioxidants [163]. Nagao et al. compared lycopene treatment (a carotenoid found in tomatoes) and vitamin C with vitamin C treatment alone, for leukoplakia [164]. No difference in clinical response rate on examination or cancer development was demonstrated between the two groups [164].

Two Indian studies have claimed carotenoid treatment results in more resolution of lesions, when compared to placebo. A pharmaceutical-sponsored trial in India compared different doses of lycopene with placebo for the resolution of leukoplakia [165]. Twenty patients took 8 mg of lycopene per day, 18 patients took 4 mg, and 18 patients took placebo. This small study, with a short 5-month follow-up, reported a statistically significant improved response (including not only complete but partial and stable responses) in patients receiving lycopene of either dose compared to placebo, for the resolution of lesions on clinical examination. Results for histopathological resolution showed a difference between the three groups, with 8 mg lycopene superior to 4 mg, which was in term superior to placebo. Development of carcinoma was not examined in this trial.

A prior study by Sankaranarayan et al. showed greater resolution of leukoplakia in patients receiving oral beta carotene (33%) compared with placebo (10%) [161]. Following cessation of treatment half of the patients who responded to beta carotene had lesion recurrence. There was no difference in progression to cancer between the two groups. Of the 15 patients who initially responded to beta carotene, two developed malignancy adjacent to the leukoplakia, 12 months after cessation of treatment [130].

There is no evidence that systemic carotenoid treatment prevents carcinogenic progression in patients with oral leukoplakia. This treatment modality should not be routinely employed for OPMDs.

Non-steroidal Anti-inflammatory Drugs

The potential for the use of non-steroidal anti-inflammatory drugs (NSAIDs) in oral premalignant lesions has been investigated following promising results of the use of NSAIDs in other types of cancer [146].

As previously stated, immunohistochemical studies have indicated that the enzyme cyclooxygenase-2 is present in both OPMD and OSCC, its presence increasing in proportion with the degree of dysplasia [142]. It has been postulated that blocking COX-2 and subsequently downregulating prostaglandin levels may hamper the carcinogenic progression of OPMDs. The use of celecoxib (a COX-2 selective non-steroidal anti-inflammatory drug) in patients with OPMD has been investigated in a randomised control trial [143]. This trial failed to demonstrate any chemopreventative effect of celecoxib nor any difference in clinical resolution of OPMD with celecoxib compared to placebo. This treatment strategy has since been abandoned.

A phase I open-label trial of acetylsalicylic acid gargle for a period of 4–6 weeks prior to excision of histologically proven oral leukoplakia has completed recruitment in the UK. Primary outcomes of PGE-1, COX-1 and COX-2 levels are yet to be reported [166].

A randomised, double-blind trial of the NSAID sulindac in the treatment of histologically suspected or confirmed index oral premalignant lesions, 12 mm or greater in size, is due for completion in June 2017 [167]. This study compares 24 weeks of oral sulindac with oral placebo, with the primary outcome measure being clinical response on oral examination and histological response (change in grade).

Oral Hypoglycaemic Agents

Metformin is a commonly prescribed oral hypoglycaemic agent. The National Cancer Institute (NCI) is currently recruiting patients for a study examining the effectiveness of oral metformin in preventing progression of oral leukoplakia or erythroplakia with mild, moderate or severe histologic dysplasia, the primary outcome being clinical response [168].

A phase IIA open-label trial of rosiglitazone (an oral thiazolidinedione hypoglycaemic agent) examining the rate of clinical response of oral premalignant lesions (leukoplakia) has completed recruitment; however published results are not yet available [169].

Biologics

Recent advances in the understanding of the biology of head and neck cancer have led to the investigation of the role of biologic agents in head and neck cancer treatment [170]. Multiple potential targets for biologic agents to treat head and neck cancer have been proposed. A recent review of the role of biologic agents in head and neck cancer listed the following potential targets: epidermal growth factor receptor (EGFR) and the ErbB family, vascular endothelial growth factor (VEGF) and its receptor (VEGFR), insulin-like growth factor 1 receptor (IGF-1R), insulin receptor (IR), histone deacetylase (HDAC), mammalian target of rapamycin (mTOR), platelet-derived growth factor receptor (PDGFR), heat-shock protein 90 (HSP90), nuclear factor-kappa B (NF-κB), aurora A or B and phosphatidylinositol 3-kinase (PIK3CA) [171].

To date, the most successful target for biologic agents has been EGFR. Overexpression of EGFR in squamous cell carcinoma of the head and neck was recognised over two decades ago [172]. Cetuximab, an epidermal growth factor receptor inhibitor, is an IgG1 antibody that avidly binds to EGFR—interfering with normal cell signalling [171].

Cetuximab is approved in Australia for the treatment of patients with HNSCC in combination with radiation therapy for locally advanced disease or in combination with platinum-based chemotherapy for recurrent and/or metastatic disease [173]. Cetuximab also has Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval for the treatment of squamous cell carcinoma of the head and neck [174, 175].

Investigation of the use of biologic agents in OPMDs has been limited to OLP. OLP is a T-cell immune-mediated disease [176] that theoretically could be managed with biologic agents to interrupt the underlying chronic inflammatory process seen in this disease. A range of biologics have been trialled for OLP [177]. So far, the experience of biological therapy for OLP does not extend beyond case series [177] and a single, open-label, prospective trial of four patients [178], with reports of significant side effects. While the theoretical opportunity for targeted biological therapy appears promising, there is yet no significant evidence that biological agents should be considered for routine therapy of OLP.

The well-recognised multistep carcinogenesis process from OPMD to malignancy provides multiple targets for biologic agents, some known and undoubtedly some yet to be discovered.

Surgical Treatment

Surgical treatment is the management strategy of choice of most specialists dealing with oral potentially malignant disorders [43, 129]. Surgical treatment of OPMDs has not been assessed in a randomised control trial that included a placebo or no treatment arm [128]. Surgical treatment options that have been proposed include surgical excision with cold steel, laser therapy and cryotherapy.

Cryotherapy

Cryotherapy destroys tissue by an application of very low temperatures, freezing tissue in situ. Cryotherapy can be performed using either an 'open' or 'closed' system [77]. Cryotherapy for the treatment of oral potentially malignant disorders was first reported in the 1970s [179]. It is not advocated for the treatment of oral potentially malignant disorders.

Cryotherapy results in the destruction of lesions without providing a specimen for further analysis. Cryotherapy does not significantly eradicate the lesion, can result in disabling contractions post-treatment and most importantly does not inhibit progression to oral carcinoma. Postoperative scarring and contracture can complicate post-treatment surveillance of the area treated [129]. In 1972, Sako et al. treated 60 patients with oral leukoplakia with cryotherapy, with disappointing results. Twelve of the 60 patients developed recurrence, and four of the 60 developed squamous cell carcinoma in the 2.5–4 years following the treatment [180].

In 2012, Kawczyk-Krupka et al. reported a non-randomised trial comparing photodynamic therapy with cryotherapy for the treatment of oral leukoplakia [181]. Prior to treatment, 'fragments of the changed mucosa were biopsied for pathological analysis', yet the authors do not report the results of these biopsies nor the inclusion/exclusion criteria based on the histopathology results. Oral leukoplakic lesions in 37 patients were treated with a contact scapular probe and refrigerated with nitrous oxide to the temperature of −89 °C, while duration and number of session varied. Over a mean follow-up period of 52 months, 89% had complete response on clinical examination, whereas 24% had recurrence. Malignant transformation rates were not reported.

Cryotherapy continues to be proposed as a treatment option for oral precancerous lesions [77, 120, 181], with reports of recurrence [65, 120, 180] and with no evidence this treatment prevents progression to carcinoma. Cryotherapy does not provide an excisional specimen for histopathological analysis, undoubtedly resulting in inadequate treatment of oral malignancies with cryotherapy. Cryotherapy should not be employed for managing OPMDs.

Cryotherapy has been described in the treatment of actinic cheilitis [154, 182]. The authors of a systemic review on actinic cheilitis treatment cite speed and ease

of application, use without anaesthesia and in-clinic use as advantages to this treatment approach [154]. Disadvantages include short-term pain and oedema, lack of standardisation of delivery, scarring and lip discoloration [154]. No studies have reported the effectiveness of cryosurgery in the treatment of actinic cheilitis.

Laser Therapy

Laser treatment has been proposed for the management of oral potentially malignant disorders, dating back to the late 1970s [183]. Laser can be used in two ways—either for vaporisation of the surface mucosa or to excise lesions. Carbon dioxide, KTP and Nd:YAG lasers have all been used in the management of oral leukoplakia [129].

Proponents of laser treatment cite the haemostatic effect of laser to be a positive of using the technique. Suturing is not required with laser, and haemostasis is maintained peri- and postoperatively. Laser therapy is said to have limited postoperative tissue contraction and scarring—which is stated to improve functionality [129, 184]. Authors report the ability to excise large areas with reduced patient morbidity and less compromise on function [129, 185]. In addition, it can be performed under local anaesthesia in an outpatient setting [186].

Drawbacks to laser surgery include prolonged wound healing and possible granuloma formation which may complicate healing [186, 187]. Histopathological confirmation of the nature of the entire OPMD remains unknown when vaporisation is used [146], which many would consider prohibitive for use of this technique, without initial confirmation of histopathology on incisional biopsy.

Overall the quality of evidence for the use of laser treatments to prevent malignant transformation of OPMDs is low. There is a heterogenous group of observational studies, using vaporisation, excision or both, with varied follow-up times. Review articles draw mainly on the results of these old, observational studies in their discussions regarding the efficacy of laser therapy in the management of OPMD [129, 186].

Studies examining the efficacy of vaporisation of OPMD using laser treatment have yielded recurrence rates of 9.7–28.9% [188–190] with malignant transformation rates between 0% and 7%, over a maximum of 60 months' follow-up [189].

Laser excision of OPMDs in two studies showed malignant transformation rates of 0% [191] and 6% [192] with a follow-up period of 24 months and 54 months, respectively. Recurrence rates for excision of OPMD using laser have been reported to be between 9.9% and 27.2% [192–194].

A further group of studies report recurrence between 8% and 38% [56, 184, 187, 195–198], with malignant transformation rates between 0% and 9% [184, 187, 195, 196], using a mix between vaporisation and excision with the CO_2 and Nd:YAG laser.

While laser therapy has regularly been examined as a single entity in the literature—in fact it is two entities, vaporisation of presumed non-malignant tissue versus laser excision of oral potentially malignant disorders allowing for further histo-

pathological assessment. It has been demonstrated over three decades ago [199], and since been widely accepted, that incisional biopsies are not truly representative of the nature of an oral potentially malignant disorder and that excision and histopathological examination of the entire lesion are required to correctly grade oral epithelial dysplasia.

Advocates state that laser surgery can only be used if cancer is excluded with biopsy. The obvious contraindication here is that incisional biopsy is only representative of the OPMD and incisional biopsy free of carcinoma in one area does not exclude carcinoma in another part of the lesion.

Thomson et al. reported the results of excisional laser surgery for 55 'oral precancerous lesions' (the majority of them being leukoplakia), which had not been previously treated and exhibited epithelial dysplasia on incisional biopsy [184]. The heterogeneity of the literature on OPMDs is again highlighted, with the inclusion by these authors of carcinoma in situ and microinvasive SCC as 'oral precancerous lesions'. Under general anaesthetic, the lesions were excised using CO_2 laser, and exposed areas were vaporised. Histopathological examination of excisional laser specimens revealed more severe disease than initial incisional biopsies in 11/55 specimens, five of which were upstaged to SCC. This elegantly demonstrates the point that laser vaporisation of OPMDs with prior non-malignant incisional biopsy will miss cases of SCC. In addition to this, four patients with a correct diagnosis of OPMD developed OSCC during follow-up.

Laser treatment of oral leukoplakia has been assessed in a randomised controlled trial, albeit in a very small cohort with no control arm [200]. Schwarz et al. compared CO_2 laser to Er:YAG, with five patients and eight lesions in each arm of the trial. Following brush cytology and DNA cytometry and histopathological examination of a representative incisional biopsy, leukoplakia was ablated with the laser, rather than excised. Half of the lesions had complete response to therapy, with the study not significantly powered to ascertain a difference between the two. Malignant transformation rates were not reported, and follow-up was short, ranging from 24 to 96 weeks.

More recently, Nammour and colleagues reported on different laser-supported surgical protocols for the treatment of oral leukoplakia specifically and undertook long-term follow-up to assess success rates [201]. They found that the surgical laser protocol respecting the complete excision of leukoplakias, in one session, by the removal of a minimum of 1 mm in lesion depth and 3 mm of surrounding healthy-like tissues offered significantly higher success rates compared to other strategies which included complete superficial vaporisation, complete excision of lesions with tissue depth of 1 and 1 mm of surrounding healthy-like tissue or complete surgical excision of large leukoplakia (more than 20 mm) performed in multiple sessions spaced by 1 month (partial surgical removal of 10 mm per session) [201].

Laser vaporisation has no role in the management of oral potentially malignant disorders. Laser excision of OPMDs provides a specimen for histopathological examination. Although the study by Nammour et al. [201] is encouraging and points to a protocol similar to that highlighted in our approach by cold steel (Fig. 11.2), there is a need for a randomised controlled trial examining the efficacy of laser exci-

sion in the management of oral potentially malignant disorders and in preventing progression to malignancy.

Vermilionectomy by carbon dioxide laser vaporisation has been described [155]. The most significant drawback of this technique is that it does not provide a specimen for histopathological analysis.

In a small series of ten patients treated in 1989, all had resolution of actinic cheilitis on clinical examination and resolution of cellular atypia on histopathological examination of a random punch biopsy at 1-year post-treatment [155]. In 1990, Zelickson et al. reported clinical resolution for all but four of 43 cases of actinic cheilitis (with preoperative moderate to severe dysplasia on histopathology), mean follow-up 20 months, with three recurrences and one progression to squamous cell carcinoma [202].

In 2009, de Godoy Peres reported postoperative histopathological outcomes of 26 patients treated for actinic cheilitis with the CO_2 laser. Using a comparative bilateral lip model, each patient had half their vermillion treated with 250 mJ and half with 350 mJ CO_2 laser. Postoperative histopathology demonstrated complete resolution of atypia in 53.8% treated with the 250 mJ laser and 61.5% with the 350 mJ [203].

In a small study of 12 patients with clinically (four of 12 patients) and histopathologically proven (eight of 12) actinic cheilitis treated with the erbium:yttrium-aluminium-garnet (Er:YAG) laser on a single occasion demonstrated no recurrences on clinical examination, mean follow-up 23 months [204]. The authors' opinion is that the Er:YAG laser anecdotally has superior evaporative efficiency, minimal thermal injury and improved healing compared to the CO_2 laser, claims which were not examined in the same study [204].

In a larger, retrospective, telephone interview follow-up study, the rate of patient-reported resolution of symptoms of actinic cheilitis was 84%, mean time to telephone follow-up was 65 months [205]. Clinical examination or histopathological resolution was not reported.

Johnson et al. report immediate post-treatment biopsy results for 14 patients treatment with CO_2 laser for AC. Each patient was treated with clinician-determined number of sequential laser passes, with interval punch biopsies—none which demonstrated residual disease. This small study recommends treatment with one or two passes of the laser, without clear evidence to support this [206].

To date, there is mixed evidence about the treatment efficacy of laser vermillionectomy and no evidence laser therapy prevents progression to invasive carcinoma.

Scalpel Excision

The European Association of Oral Medicine protocol for the management of oral leukoplakia and erythroplakia recommends surgery, either by cold knife or by laser, as the treatment modality of choice for leukoplakia and erythroplakia [43]. A 2007 survey of diplomates of the American Board of Oral Medicine [159] showed that

cold steel surgical excision was the preferred method of management of oral potentially malignant disorders with this approach suggested by 69% of responders.

The efficacy of surgical excision of OPMD has never been investigated with a randomised controlled trial that compares it to placebo or no treatment. The studies we have in order to assess the efficacy of this treatment are observational studies, performed since the 1960s.

The most obvious and important advantage of scalpel excision of OPMD is that the whole lesion is available for histopathological examination. This highlights cold steel excision of OPMDs as an effective diagnostic tool, as histopathologic examination is still the gold standard diagnostic test for these lesions [43].

It has been well described that a subset of OPMDs excised following an incisional biopsy have been found to contain squamous cell carcinoma on histopathology, with rates of up to 10% reported [184, 197, 207]. It has also been shown that there is intralesional molecular heterogeneity within a single leukoplakic lesion, providing evidence for excision of the whole lesion and proving an explanation for disparity between incisional and excisional biopsy results of OPMDs [208]. Aside from the obvious question as to whether surgical excision prevents malignant transformation of OPMDs, surgical excision of OPMDs can be justified on the basis of diagnosis alone [209].

A cited drawback to cold steel excision of OPMDs is postoperative wound contraction. Large defects may need reconstruction—and it has been proposed that covering an area of excised OPMD with a flap or graft may hide recurrence of malignant transformation in that area [187]. More recently however, the coverage of surgical beds with synthetic resorbable material such as 3D collagen matrices (such as Geistlich Mucograft® or Orthocell CelGro®) has facilitated wound closure without wound contraction, thus permitting excision of larger areas of OPMD (particularly leukoplakia). These simple reconstructive approaches augment surgical excision of OPMD by cold steel (Fig. 11.5). Studies assessing removal of OPMD with electrocautery or diathermy are lacking.

Acknowledgement and consideration of the quality of the evidence for and against surgical excision of oral potentially malignant disorders must precede discussion of the possible answers provided by the evidence. There is no high-level evidence for or against surgical excision of OPMDs to prevent malignant transformation. Attempts to compare groups treated with surgical excision of OPMD with groups under surveillance are inherently flawed. It is more than reasonable to assume that patients with more concerning clinical presentations (patient factors, lesion factors, history, etc.) would be selected for intervention, while their counterparts deemed to be ‘low risk’ by the treating clinician would be selected for observation. It is easy to see that if we compare malignant transformation in patients selected for intervention with patients selected for observation without randomisation; observed differences in these groups will not provide meaningful information to help answer the question of which treatment strategy is superior.

Observational studies form the bulk of the literature that seeks to answer the question—does surgical excision of oral potentially malignant disorders prevent malignant transformation?

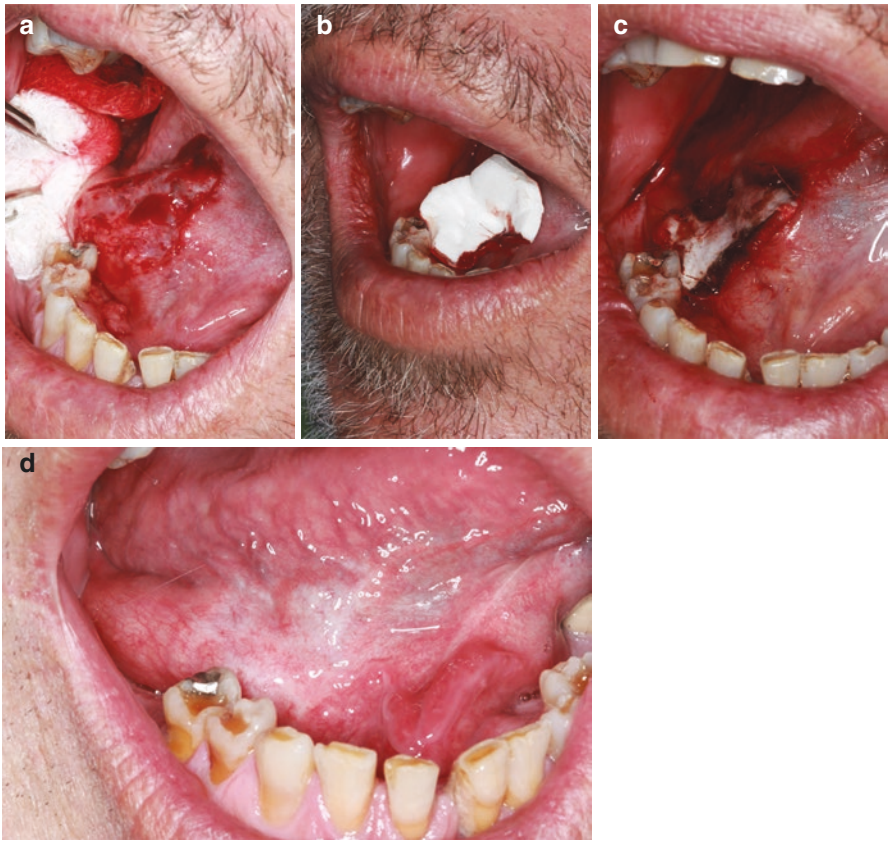


Fig. 11.5 (**a**) Surgical bed following removal of non-homogenous leukoplakia of the floor of the mouth resected under local anaesthesia. (**b**) Synthetic resorbable material matrix (Geistlich Mucograft®) cut to the size of surgical defect. (**c**) Synthetic resorbable material matrix (Geistlich Mucograft®) sutured into position. (**d**) Surgical site following healing demonstrates wound closure without scarring or contraction. Histopathology confirmed moderate oral epithelial dysplasia. This simple reconstructive approach augments surgical excision of leukoplakia by cold steel permitting excision of larger areas as detailed in management guidelines (Fig. 11.2)

In 1967, Einhorn et al. concluded from the findings of an observational trial that there was no evidence that the incidence of oral carcinoma could be diminished by surgical removal of oral leukoplakia [49]. Since then further observational studies have drawn the same conclusion [54, 98, 115, 210], while a single observational study reported that excisional biopsy of oral leukoplakia prevented malignant transformation [211].

Criteria for reporting oral leukoplakia have changed several times since these early observational studies were first reported. This further complicates comparison and grouping of study findings [128]. Reporting within studies themselves is often unclear and not transferable to the clinical situation. One observational study

grouped OPMDs proven to be carcinoma in situ with the OPMDs, eventually including them in the 12% malignant transformation rate and then commenting they accounted for many of the 'malignant transformations' in the group. This is an example of design flaws that permeate the literature on this topic [98].

Mehanna et al. performed a systematic review and meta-analysis examining the treatment and follow-up of oral dysplastic lesions [113]. Fourteen prospective and retrospective studies were included. When the published data was pooled, 992 patients with oral dysplasia were included. This study design pooled results from heterogenous trials—with different inclusion criteria, designs, treatment and follow-up interval and duration. The authors reported a statistically significant difference in the malignant transformation rate, with a higher transformation rate for patients who did not undergo surgical excision compared with those who did—14.6% to 5.4%. This result should be interpreted with caution given pooling different studies with marked differences is unlikely to provide a meaningful result.

Despite this, at present there is no convincing evidence that surgical excision of OPMDs prevents malignant transformation. Surgical excision with a margin of normal oral mucosa is recommended, as a diagnostic tool alone. There is an urgent need for a randomised control clinical trial examining the effectiveness of surgical excision of OPMDs.

An additional matter to consider with surgical excision or indeed laser excision is the extent of the surgical margin of the lesion. Molecular aberrations exist in 'normal'-appearing mucosa adjacent to clinically apparent lesions, and this may account for recurrence of surgically treated lesions [209, 212]. It is not possible to precisely determine the margin of OPMDs. For these reasons, excision should be a distance from the edge of the lesion. Optical adjunctive devices such as optical fluorescence imaging (e.g. VELscope®, LED Dental; Bio/Screen®, AdDent Inc; Identafi®, StarDental) and narrowband imaging (NBI; Olympus®) are emerging as important tools to improve the accuracy of margin delineation (Fig. 11.6) [213]. These devices demonstrate lesions extending beyond the clinical 'margin' present on examination with the naked eye or white light alone. A prospective gene-profiling study examining the utility of NBI in determining margins for OSCC resection demonstrated the presence of molecular aberrations in the white light margin surrounding OSCC, with more abnormalities in margins delineated with white light as compared to NBI [213]. In a retrospective, case-control observational study, Poh et al. report a reduced rate of local recurrence in high-grade OPMDs when fluorescence utilisation was used as part of preoperative margin delineation [214]. Adjunctive diagnostic devices, such as NBI and direct autofluorescence, typically show lesions to be larger under these devices when compared to the naked eye [215].

At present, there are no guidelines recommending the width of the surgical margins [15, 43]. The European Association of Oral Medicine suggests most clinicians would excise the lesion with a margin of a 'few millimetres' of macroscopically normal tissue [43]. It should be noted that a macroscopically normal margin may not be possible with widespread lesions or lesions over anatomically sensitive sites, such as salivary gland ducts [134]. Our own protocol (Fig. 11.2) recommends com-

Fig. 11.6 (**a**) VELscope Vx® (LED Dental) fluorescence visualisation device. (**b**) Bio/Screen® (AdDent Inc) fluorescence device. (**c**) Identafi® (StarDental) multispectral visualisation device. (**d**) Narrow Band Imaging (Olympus®) endoscopic unit

plete removal of lesions (whenever possible) with a clear margin of normal tissue (1–2 mm) achieved with the use of adjunctive optical devices (optical fluorescence imaging or narrowband imaging) and complete closure.

Cold steel vermilionectomy has been described as treatment for actinic cheilitis (Fig. 11.7) [154]. Simple vermilionectomy involves vermilion epidermal resection, with glandular and orbicularis oris muscle resected in a modified vermilionectomy [154]. Vermilionectomy is operator dependent [154], with a multitude of techniques being described. It is the only treatment of actinic cheilitis that provides a specimen for histopathological analysis. Menta Simonsen Nico et al. compared initial punch biopsy diagnoses of actinic cheilitis with subsequent histopathological analysis of cold steel vermilionectomy specimens in a series of 20 patients [216]. In 40% of the cases, the complete specimen yielded more severe histopathological alterations when compared with the original punch biopsy. In one of the 20 cases, invasive

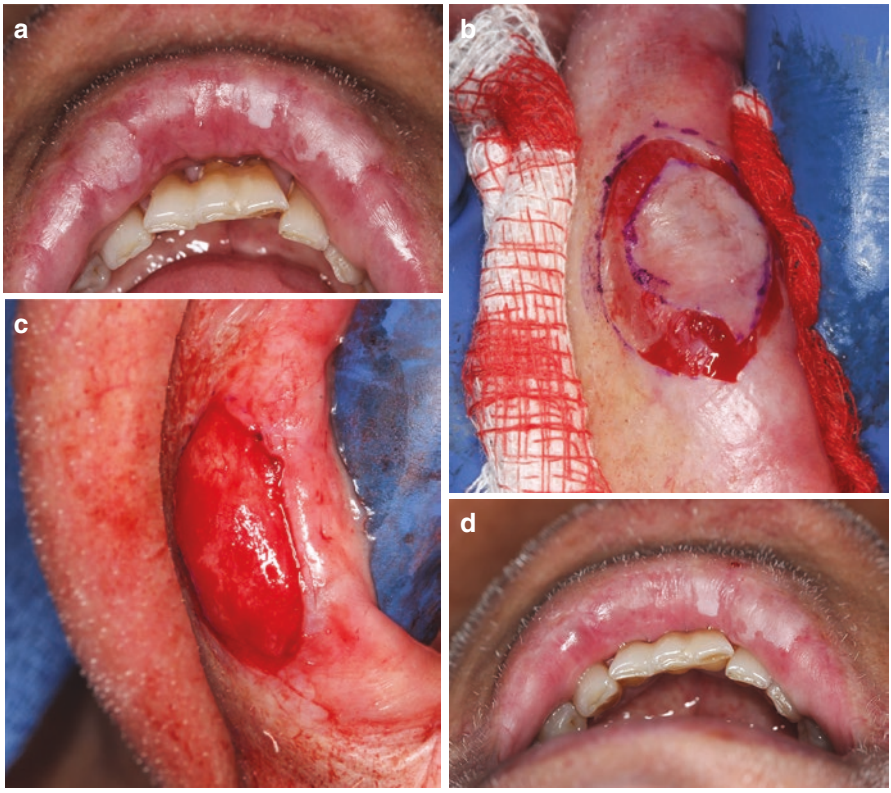


Fig. 11.7 (**a**) Actinic cheilitis in an elderly male with a history of sun exposure presenting on the lower lip with widespread non-homogenous keratotic white changes. The lesion on the lower left lip was removed with cold steel excision (**b**) with a margin of normal tissue down to underlying connective tissue (**c**) and closed with direct primary closure. (**d**) Clinical appearance of the lower left lip 2 weeks postsurgical excision and direct primary closure. Healing was uneventful with good aesthetic outcome. Histopathology revealed orthokeratosis with no epithelial dysplasia

squamous cell carcinoma was diagnosed on the analysis of the vermilionectomy specimen.

In 1989, a small randomised trial of ten patients treated with vermilionectomy reported clinical and histopathological resolution of actinic cheilitis with this treatment in all patients [155]. Satorres Nieto et al. reported a series of 41 patients with actinic cheilitis treated with cold steel vermilionectomy. Of these, none had evidence of clinical recurrence at minimum 6 months' follow-up [217].

Following vermilionectomy, multiple techniques for closure of the defect have been described, including direct primary closure and mucosal advancement flaps with or without tissue undermining and pedicled buccal mucosal flaps [217–219].

Vermilionectomy with cold steel appears to be the preferred method for definitive treatment of actinic cheilitis with good outcomes, in addition to having the distinct advantage of being the only proposed treatment method for actinic cheilitis that provides a specimen for histopathological analysis (Fig. 11.7).

Photo Dynamic Therapy

Over a century ago, researchers have noted that they could induce cell death using a combination of light and chemicals [220]. Modern photodynamic therapy (PDT) exerts its effect on tissues and cells utilising three components. The first is a 'photosensitiser'—a molecule that localises to target tissues. The second is a light of a specific wavelength that will activate the photosensitiser. The third is oxygen. In the presence of oxygen, the first two components generate reactive oxygen species and other radicals [220], and these products damage tumour cells and their vasculature and generate an immune response.

A recent systematic review of 12 studies concluded that PDT was a useful treatment strategy in the management of oral premalignant lesions [221]. Pooled studies cited in the systematic review showed a recurrence rate between 0% and 36%.

In a large prospective study, 147 patients with oral dysplasia or carcinoma in situ were treated with PDT [222]. The grouping of carcinoma in situ with oral potentially malignant lesions is an example of the heterogeneity of the literature on the topic. In this trial, 81% of patients had complete response to PDT at 5 years, with one or more applications. The recurrence rate was 12%. Eleven patients in the cohort (7.5%) had subsequent malignant transformation; patients with carcinoma in situ are more likely to progress.

Proponents of PDT cite ease of administration in the outpatient setting [223], good functional and cosmetic outcomes [130] and low systemic toxicity as positives of PDT [130]. There are drawbacks to using PDT however. Patients must avoid sunlight for 2–3 weeks post-application, which restricts the routine use of PDT for most patients. PDT of OPMD has an unacceptable drawback, similar to the use of laser vaporisation of OPMDs, not permitting histopathological examination of the whole lesion. It is known that a subset of patients diagnosed with OPMD on incisional biopsy will be found to have carcinoma on examination of the complete lesion following excision. The efficacy of PDT has not been compared with placebo, and at present there is no convincing evidence PDT is superior to placebo in preventing malignant progression. There is however a phase III, randomised, double-blind trial underway in Vienna currently recruiting patients with oral leukoplakia and oral lichen planus, comparing the efficacy of PDT and aminolaevulinic acid with placebo [224]. The primary outcome measure is the area affected by OPMD, as measured by a caliper. At this stage however, PDT is not currently recommended for the management of OPMDs.

Photodynamic therapy has been proposed as a treatment for actinic cheilitis [225]. Four studies have examined the efficacy of PDT in treating actinic cheilitis, comparing pre- and post-treatment histopathology. The first treated ten patients with 5-aminolaevulinic acid-based photodynamic therapy, reporting an 80% histopathological cure rate at 3 months [226]. The same group reported an 18-month follow-up study of the same PDT technique, with a cure rate of 65.4% on histopathological analysis [227]. Berking et al. used methyl-aminoxopentanoate-based PDT (MAL-PDT), reporting 38% histopathological cure rate, also at 3 months [228]. The fourth study prospectively examined treatment with fractionated photodynamic therapy,

two treatment doses on a single day. Follow-up was 18 months, with half of the ten patients having histopathologically proven persistent or recurrent actinic cheilitis [229].

In a randomised controlled trial of 33 patients, Choi et al. compared routine two-session MAL-PDT with pretreatment with fractional laser resurfacing using the erbium:yttrium-aluminium-garnet (Er:YAG) laser, followed by MAL-PDT in the comparator group [230]. Pretreatment with the Er:YAG laser resulted in a complete histological and clinical response of 79% at 12 months, compared to 26% in the routine MAL-PDT group, with a statistically significant difference between the two groups. The use of Er:YAG pretreatment PDT has not been further investigated beyond this small trial.

Kim et al. investigated multiple sessions of PDT as treatment for actinic cheilitis. After an average of 4.6 treatments, five of ten patients showed complete clinical response. Two later recurred, yielding a response rate of 30%, even with repeated therapies [231].

Photodynamic therapy has not been demonstrated to be an efficacious treatment for actinic cheilitis.

Other

Limiting sun exposure to actinic cheilitis-affected areas of the lower lip is thought to prevent progression of actinic cheilitis to carcinoma, but this has not been demonstrated in well-designed follow-up studies [103].

Follow-Up

As with the prementioned aspects of the management of OPMDs, there is no high-level evidence to suggest appropriate follow-up of patients with oral potentially malignant disorders. There are no randomised controlled trials examining the topic [128].

Patients with OPMDs should be monitored closely. The efficacy of close monitoring however in improving patient outcomes has not been demonstrated [117].

Malignant transformation can occur over a long period of time following diagnosis of an OPMD [210]. In a large observational study of oral potentially malignant disorders in 1458 patients, the average interval from diagnosis to malignant transformation was 43 months [232]. The delay in progression of OPMDs has led to the recommendation of lifelong follow-up of patients with and without dysplasia [117]. There exists no consensus about appropriate follow-up intervals. The European Association of Oral Medicine suggests follow-up at intervals of 6 months for non-dysplastic leukoplakia (low-risk lesions) and 3 months for dysplastic leukoplakic lesions (higher-risk lesions) [43]. Field et al. recommend long-term review of patients with moderate to severe OED 1 month post-surgery and then 3, 6 and 12

monthly depending on clinical assessment, histopathology and previous history [16]. In cases of mild OED, patients are reviewed 6 and then 12 monthly for a period of 5 years and then discharged to primary care practitioners depending on past dental history and attendance, patient preference and liaison with the patient's dental practitioner [16]. Our recommendations for long-term surveillance of patients with OPMD are summarised in Fig. 11.2. Regular long-term follow-up of patients with OLP is necessary with the frequency of clinical reviews based on clinical parameters such as the presence of erosive or ulcerative lesions [57].

The cost-effectiveness of long-term follow-up of patients with OPMDs is not known [210]. To optimise resource allocation, it has been proposed that long-term follow-up be shared between primary and secondary healthcare providers, with specialists supporting their colleagues [129].

Further research is needed to examine the cost-effectiveness of lifelong surveillance and to determine whether it facilitates earlier diagnosis of malignant transformation and whether it improves long-term patient outcomes.

Multidisciplinary Approach to Patient Care

Oral potentially malignant disorders, representing a diverse group of lesions and conditions, both in terms of etiopathogenesis and clinical presentations, are managed by a variety of clinicians with expertise in medicine, including oral medicine, surgery and pathology. Depending on the nature and extent of the particular disorder, the treating clinician (or clinicians) may range from oral medicine specialists, dermatologists and immunologists to oral and maxillofacial surgeons, ENT and/or plastic surgeons [233].

While at present there is a distinct lack of evidence-based guidelines regarding the optimal management of OPMDs [16], it is generally accepted that complete excision is probably the ideal treatment option, and this is particularly the case when a histopathological diagnosis of oral epithelial dysplasia is present—the strongest predictor of future malignant transformation in an OPMD [147]. It is therefore not surprising that a recent British survey intended to ascertain treatment protocols, targeting clinicians who treat OPMDs from oral and maxillofacial surgery, oral medicine, ENT and plastic surgery, identified the majority of participants to be oral and maxillofacial surgeons (71%), followed by ENT (19%) [14].

It has long been recognised that the optimal management of many complex diseases, including head and neck cancer, requires a multidisciplinary treatment approach to ensure that optimal patient outcomes are achieved and is now considered the standard of care [16, 25, 234]. The benefits of a multidisciplinary approach in the management of OPMDs are also becoming increasingly apparent [16]. Given the diverse presentations and potential complexities associated with the treatment of individuals with these disorders, it is clear that no one healthcare provider can be expected to hold all the necessary skills and expertise to ensure optimal treatment of the affected individual. A multidisciplinary team assessment and discussion, as can

occur in the setting of a head and neck cancer clinic, are far more likely to ensure that all factors relevant to treatment planning are considered and that individuals are offered the best treatment advice, which is not limited or prejudiced by the skill or expertise of a single clinician [234]. A multidisciplinary approach to the management of patients with OPMD, particularly those exhibiting oral epithelial dysplasia, is therefore currently considered best practice [16].

Conclusion

There are no truly effective treatment options for OPMDs. Treatment of OPMDs is complicated by the heterogeneity of pathologies included in its definition, their underlying etiopathogenesis and different approaches to managing symptoms or undertaking definitive treatment. Even if one considers treatment options for oral leukoplakia (the most common OPMD), then the heterogeneity and deficiency in study design, limits the usefulness of current literature for determination of evidence-based best practice.

To date, the best treatment option for OPMD appears to be cold steel removal with a margin of macroscopically normal oral mucosa, particularly for oral leukoplakia and erythroplakia. Additionally, management of infectious and inflammatory components of OPMDs should be undertaken as appropriate, with regular follow-up. Management of OPMDs, especially those in high-risk patients, is best achieved in a multidisciplinary setting. With greater knowledge and more well-designed studies around the role and application of optical adjuncts, these may become valuable for clinicians to use not only in early detection of OPMD but also their management.

References

1. Kramer IR, et al. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol. 1978;46(4):518–39.
2. Axell T, et al. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21 1994. International Collaborative Group on Oral White Lesions. J Oral Pathol Med. 1996;25(2):49–54.
3. Mortazavi H. Oral potentially malignant disorders: an overview of more than 20 entities. J Dent Res Dent Clin Dent Prospects. 2014;8(1):6–14.
4. Bouquot JE, Speight PM, Farthing PM. Epithelial dysplasia of the oral mucosa—diagnostic problems and prognostic features. Oral Oncol. 2006;12:11–21.
5. Bombeccari GP, et al. Oral lichen planus and malignant transformation: a longitudinal cohort study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112(3):328–34.
6. Silverman S Jr, Gorsky M, Lozada-Nur F. A prospective follow-up study of 570 patients with oral lichen planus: persistence, remission, and malignant association. Oral Surg Oral Med Oral Pathol. 1985;60(1):30–4.

7. Landini G, et al. The reported rates of transformation of oral lichen planus. J Oral Maxillofac Surg Med Pathol. 2014;26:213–22.
8. Al-Hashimi I, et al. Oral lichen planus and oral lichenoid lesions: diagnostic and therapeutic considerations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103(Suppl):S25.e1–12.
9. van der Meij EH, et al. A review of the recent literature regarding malignant transformation of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;88(3):307–10.
10. Ismail SB, Kumar SK, Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci. 2007;49(2):89–106.
11. Calcaianu N, et al. Surgical attitude in premalignant lesions and malignant tumors of the lower lip. J Med Life. 2015;8(1):109–11.
12. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008;37(1):1–10.
13. Dost F, Do L, Farah CS. Lesion Evaluation, Screening and Identification of Oral Neoplasia Study: an assessment of high-risk Australian populations. Community Dent Oral Epidemiol. 2016;44(1):64–75.
14. Thomson PJ, et al. To treat…or not to treat? Clinicians' views on the management of oral potentially malignant disorders. Br J Oral Maxillofac Surg. 2015;53(10):1027–31.
15. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45(4-5):317–23.
16. Field EA, et al. The management of oral epithelial dysplasia: the Liverpool algorithm. Oral Oncol. 2015;51(10):883–7.
17. Epstein JB, et al. Oral complications of cancer and cancer therapy: from cancer treatment to survivorship. CA Cancer J Clin. 2012;62(6):400–22.
18. Yu GP, et al. Non-cancer-related deaths from suicide, cardiovascular disease, and pneumonia in patients with oral cavity and oropharyngeal squamous carcinoma. Arch Otolaryngol Head Neck Surg. 2012;138(1):25–32.
19. Tadakamadla J, Kumar S, Johnson NW. Quality of life in patients with oral potentially malignant disorders: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119(6):644–55.
20. Flaitz CM, Carlin N. Living in limbo: ethics and experience in a conversation about persistent oral lesions. Tex Dent J. 2013;130(8):692–701.
21. Lin HY, et al. Unmet information needs and clinical characteristics in patients with precancerous oral lesions. Eur J Cancer Care. 2015;24(6):911–9.
22. More CB, et al. Proposed clinical classification for oral submucous fibrosis. Oral Oncol. 2012;48(3):200–2.
23. Tilakaratne WM, et al. Intralesional corticosteroids as a treatment for restricted mouth opening in oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):224–31.
24. Lopez-Jornet P, Camacho-Alonso F. Quality of life in patients with oral lichen planus. J Eval Clin Pract. 2010;16(1):111–3.
25. Rhodus NL, et al. Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone. Oral Dis. 2006;12(2):112–6.
26. Ekanayaka RP, Tilakaratne WM. Oral submucous fibrosis: review on mechanisms of malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):192–9.
27. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; present concepts of management. Oral Oncol. 2010;46(6):423–5.
28. Nair DR, et al. Oral cancer: premalignant conditions and screening--an update. J Cancer Res Ther. 2012;8(Suppl 1):S57–66.
29. McGrath C, et al. Patient-centred outcome measures for oral mucosal disease are sensitive to treatment. Int J Oral Maxillofac Surg. 2003;32(3):334–6.
30. Ni Riordain R, McCreary C. Further reliability and responsiveness of the Chronic Oral Mucosal Diseases Questionnaire. Oral Dis. 2012;18(1):60–6.
31. Tabolli S, et al. Quality of life and psychological problems of patients with oral mucosal disease in dermatological practice. Dermatology. 2009;218(4):314–20.

32. Hegarty AM, et al. Fluticasone propionate spray and betamethasone sodium phosphate mouthrinse: a randomized crossover study for the treatment of symptomatic oral lichen planus. J Am Acad Dermatol. 2002;47(2):271–9.
33. Li M, He SL. Reliability and validity of the Chinese version of the chronic oral mucosal diseases questionnaire. J Oral Pathol Med. 2013;42(2):194–9.
34. Liu LJ, et al. Generic and oral quality of life is affected by oral mucosal diseases. BMC Oral Health. 2012;12:2.
35. Karbach J, et al. Oral health-related quality of life of patients with oral lichen planus, oral leukoplakia, or oral squamous cell carcinoma. J Oral Maxillofac Surg. 2014;72(8):1517–22.
36. Ni Riordain R, McCreary C. Validity and reliability of a newly developed quality of life questionnaire for patients with chronic oral mucosal diseases. J Oral Pathol Med. 2011;40(8):604–9.
37. Rimal J, Shrestha A. Validation of nepalese oral health impact profile14 and assessment of its impact in patients with oral submucous fibrosis in Nepal. J Nepal Health Res Counc. 2015;13(29):43–9.
38. Bassim CW, et al. Validation of the National Institutes of Health chronic GVHD Oral Mucosal Score using component-specific measures. Bone Marrow Transplant. 2014;49(1):116–21.
39. Carpenter PA, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: V. The 2014 ancillary therapy and supportive care working group report. Biol Blood Marrow Transplant. 2015;21(7):1167–87.
40. Farah CS, et al. Oral cancer and oral potentially malignant disorders. Int J Dent. 2014;2014:853479.
41. Otero-Rey EM, et al. Malignant transformation of oral lichen planus by a chronic inflammatory process. Use of topical corticosteroids to prevent this progression? Acta Odontol Scand. 2014;72(8):570–7.
42. Liu W, et al. Malignant potential of oral and labial chronic discoid lupus erythematosus: a clinicopathological study of 87 cases. Histopathology. 2011;59(2):292–8.
43. Diz P, et al. Oral leukoplakia and erythroplakia: a protocol for diagnosis and management. EAOM - Diagnostic and therapeutic protocols. 2011.
44. Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. J Oral Pathol Med. 2016;45(3):155–66.
45. Dost F, et al. A retrospective analysis of clinical features of oral malignant and potentially malignant disorders with and without oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(6):725–33.
46. Dost F, et al. Malignant transformation of oral epithelial dysplasia: a real-world evaluation of histopathologic grading. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117(3):343–52.
47. Mays JW, et al. Oral chronic graft-versus-host disease: current pathogenesis, therapy, and research. Oral Dis. 2013;19(4):327–46.
48. Kerr AR, et al. A systematic review of medical interventions for oral submucous fibrosis and future research opportunities. Oral Dis. 2011;17(Suppl 1):42–57.
49. Einhorn J, Wersall J. Incidence of oral carcinoma in patients with leukoplakia of the ora mucosa. Cancer. 1967;20:2189–93.
50. Banoczy J, Sugar L. Longitudinal studies in oral leukoplakias. J Oral Pathol. 1972;1(6):265–72.
51. Gupta PC, et al. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. Cancer. 1989;63(11):2247–52.
52. Roed-Petersen B. Cancer development in oral leukoplakia follow-up of 331 patients. J Dent Res. 1971;80:711.
53. Lind PO. Malignant transformation in oral leukoplakia. Scand J Dent Res. 1987;95(6):449–55.
54. Schepman KP, et al. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol. 1998;34:270–5.
55. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984;53(3):563–8.

56. Abadie WM, et al. Optimal management of proliferative verrucous leukoplakia: a systematic review of the literature. Otolaryngol Head Neck Surg. 2015;153(4):504–11.
57. Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: a systematic review. J Am Dent Assoc. 2014;145(1):45–56.
58. Gonzalez-Moles MA, Scully C, Gil-Montoya JA. Oral lichen planus: controversies surrounding malignant transformation. Oral Dis. 2008;14(3):229–43.
59. Smith LW, et al. Oral cancer and precancerous lesions in 57,518 industrial workers of Gujarat, India. Indian J Cancer. 1975;12(2):118–23.
60. Axell T. Occurence of leukoplakia and some other oral white lesions among 20,333 adult Swedish people. Community Dent Oral Epidemiol. 1987;15:46–51.
61. Lim K, et al. Opportunistic screening for oral cancer and precancer in general dental practice: results of a demonstration study. Br Dent J. 2003;194(9):497–502. discussion 493.
62. Banoczy J, Csiba A. Occurrence of epithelial dysplasia in oral leukoplakia. Analysis and follow-up study of 12 cases. Oral Surg Oral Med Oral Pathol. 1976;42(6):766–74.
63. Pindborg JJ, Roed-Peterson B, Renstrup G. Role of smoking in floor of the mouth leukoplakias. J Oral Pathol. 1972;1(1):22–9.
64. Banoczy J. Follow-up studies in oral leukoplakia. J Maxillofac Surg. 1977;5(1):69–75.
65. Ho PS, et al. Malignant transformation of oral potentially malignant disorders in males: a retrospective cohort study. BMC Cancer. 2009;9:260.
66. Ho MW, et al. The clinical determinants of malignant transformation in oral epithelial dysplasia. Oral Oncol. 2012;48(10):969–76.
67. Hamadah O, Thomson PJ. Factors affecting carbon dioxide laser treatment for oral precancer: a patient cohort study. Lasers Surg Med. 2009;41(1):17–25.
68. Hashibe M, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev. 2009;18(2):541–50.
69. Munoz AA, et al. Behavior of oral squamous cell carcinoma in subjects with prior lichen planus. Otolaryngol Head Neck Surg. 2007;136(3):401–4.
70. Fioretti F, et al. Risk factors for oral and pharyngeal cancer in never smokers. Oral Oncol. 1999;35(4):375–8.
71. Turati F, et al. A meta-analysis of alcohol drinking and oral and pharyngeal cancers: results from subgroup analyses. Alcohol Alcohol. 2013;48(1):107–18.
72. Shiu MN, Chen TH. Impact of betel quid, tobacco and alcohol on three-stage disease natural history of oral leukoplakia and cancer: implication for prevention of oral cancer. Eur J Cancer Prev. 2004;13(1):39–45.
73. Bauer UE, et al. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. Lancet. 2014;384(9937):45–52.
74. McCullough MJ, Farah CS. The role of alcohol in oral carcinogenesis with particular reference to alcohol-containing mouthwashes. Aust Dent J. 2008;53(4):302–5.
75. Currie S, Farah CS. Alcohol-containing mouthwash and oral cancer risk: a review of current evidence. OA Alcohol. 2014;2(1):4.
76. Gandini S, et al. Mouthwash and oral cancer risk quantitative meta-analysis of epidemiologic studies. Ann Agric Environ Med. 2012;19(2):173–80.
77. Ahrens W, et al. Oral health, dental care and mouthwash associated with upper aerodigestive tract cancer risk in Europe: the ARCAGE study. Oral Oncol. 2014;50(6):616–25.
78. Radoï L, et al. Family history of cancer, personal history of medical conditions and risk of oral cavity cancer in France: the ICARE study. BMC Cancer. 2013;13(1):560.
79. Brown LM, et al. Family cancer history and susceptibility to oral carcinoma in Puerto Rico. Cancer. 2001;92:2102.
80. Foulkes WD, et al. Family history of cancer is a risk factor for squamous cell carcinoma of the head and neck in Brazil: a case–control study. Int J Cancer. 1995;63:769.
81. Garavello W, et al. Family history and the risk of oral and pharyngeal cancer. Int J Cancer. 2008;122:1827.

82. Negri E, et al. Family history of cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Int J Cancer. 2009;124(2):394–401.
83. Dost F, Ford PJ, Farah CS. Heightened risk of second primary carcinoma of the head and neck following cervical neoplasia. Head Neck. 2014;36(8):1132–7.
84. Trizna Z, Schantz SP. Hereditary and environmental factors associated with risk and progression of head and neck cancer. Otolaryngol Clin North Am. 1992;25(5):1089–103.
85. Jefferies S, et al. The role of genetic factors in predisposition to squamous cell cancer of the head and neck. Br J Cancer. 1999;79(5-6):865–7.
86. Shridhar K, et al. Single nucleotide polymorphisms as markers of genetic susceptibility for oral potentially malignant disorders risk: review of evidence to date. Oral Oncol. 2016;61:146–51.
87. Shridhar K, et al. DNA methylation markers for oral pre-cancer progression: a critical review. Oral Oncol. 2016;53:1–9.
88. Lee YC, et al. Active and involuntary tobacco smoking and upper aerodigestive tract cancer risks in a multicenter case-control study. Cancer Epidemiol Biomarkers Prev. 2009;18(12):3353–61.
89. Paget-Bailly S, Cyr D, Luce D. Occupational exposures to asbestos, polycyclic aromatic hydrocarbons and solvents, and cancers of the oral cavity and pharynx: a quantitative literature review. Int Arch Occup Environ Health. 2012;85(4):341–51.
90. Tarvainen L, et al. Cancer of the mouth and pharynx, occupation and exposure to chemical agents in Finland [in 1971-95]. Int J Cancer. 2008;123(3):653–9.
91. Schildt EB, et al. Occupational exposures as risk factors for oral cancer evaluated in a Swedish case-control study. Oncol Rep. 1999;6(2):317–20.
92. Tsai KY, et al. Environmental heavy metal as a potential risk factor for the progression of oral potentially malignant disorders in central Taiwan. Cancer Epidemiol. 2017;47:118–24.
93. Tortorici S, et al. Prevalence and distribution of oral mucosal non-malignant lesions in the western Sicilian population. Minerva Stomatol. 2016;65(4):191–206.
94. Junqueira JL, et al. Actinic cheilitis among agricultural workers in Campinas, Brazil. Commun Dent Health. 2011;28(1):60–3.
95. Ferreira AM, et al. Prevalence and factors associated with oral potentially malignant disorders in Brazil's rural workers. Oral Dis. 2016;22(6):536–42.
96. de Souza Lucena EE, et al. Prevalence and factors associated to actinic cheilitis in beach workers. Oral Dis. 2012;18(6):575–9.
97. Kramer IR, El-Labban N, Lee KW. The clinical features and risk of malignant transformation in sublingual keratosis. Br Dent J. 1978;144(6):171–80.
98. Holmstrup P, et al. Long-term treatment outcome of oral premalignant lesions. Oral Oncol. 2006;42(5):461–74.
99. Coombes D, Cascarini L, Booth PW. Carcinoma of the midline dorsum of the tongue. Br J Oral Maxillofac Surg. 2008;46(6):485–6.
100. Saito T, et al. High malignant transformation rate of widespread multiple oral leukoplakias. Oral Dis. 1999;5(1):15–9.
101. Hamadah O, Goodson ML, Thomson PJ. Clinicopathological behaviour of multiple oral dysplastic lesions compared with that of single lesions. Br J Oral Maxillofac Surg. 2010;48(7):503–6.
102. van der Waal I. Oral potentially malignant disorders: is malignant transformation predictable and preventable? Med Oral Patol Oral Cir Bucal. 2014;19(4):e386–90.
103. Savage NW, McKay C, Faulkner C. Actinic cheilitis in dental practice. Aust Dent J. 2010;55(1 Suppl):78–84.
104. Krishnan L, et al. Inter- and intra-observer variability in three grading systems for oral epithelial dysplasia. J Oral Maxillofac Pathol. 2016;20(2):261–8.
105. Kujan O, et al. Why oral histopathology suffers inter-observer variability on grading oral epithelial dysplasia: an attempt to understand the sources of variation. Oral Oncol. 2007;43(3):224–31.

106. Wang YY, et al. Malignant transformation in 5071 southern Taiwanese patients with potentially malignant oral mucosal disorders. BMC Oral Health. 2014;14:99.
107. Krutchkoff DJ, Eisenberg E. Lichenoid dysplasia: a distinct histopathologic entity. Oral Surg Oral Med Oral Pathol. 1985;60(3):308–15.
108. van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med. 2003;32(9):507–12.
109. Bornstein MM, et al. Oral lichen planus and malignant transformation: a retrospective follow-up study of clinical and histopathologic data. Quintessence Int. 2006;37(4):261–71.
110. van der Meij EH, Mast H, van der Waal I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective five-year follow-up study of 192 patients. Oral Oncol. 2007;43(8):742–8.
111. Zhang L, et al. Should severe epithelial dysplasia be treated? Oral Oncol. 2016;60:125–9.
112. Arnaoutakis D, et al. Recurrence patterns and management of oral cavity premalignant lesions. Oral Oncol. 2013;49(8):814–7.
113. Mehanna HM, et al. Treatment and follow-up of oral dysplasia - a systematic review and meta-analysis. Head Neck. 2009;31(12):1600–9.
114. Bremmer JF, et al. Prognostic value of DNA ploidy status in patients with oral leukoplakia. Oral Oncol. 2011;47(10):956–60.
115. Brouns ER, et al. DNA ploidy measurement in oral leukoplakia: different results between flow and image cytometry. Oral Oncol. 2012;48(7):636–40.
116. Sen S. Aneuploidy and cancer. Curr Opin Oncol. 2000;12(1):82–8.
117. Bradley G, et al. Abnormal DNA content in oral epithelial dysplasia is associated with increased risk of progression to carcinoma. Br J Cancer. 2010;103(9):1432–42.
118. Sperandio M, et al. Predictive value of dysplasia grading and DNA ploidy in malignant transformation of oral potentially malignant disorders. Cancer Prev Res (Phila). 2013;6(8):822–31.
119. Sitheeque MA, Samaranayake LP. Chronic hyperplastic candidosis/candidiasis (candidal leukoplakia). Crit Rev Oral Biol Med. 2003;14(4):253–67.
120. Gainza-Cirauqui ML, et al. Production of carcinogenic acetaldehyde by Candida albicans from patients with potentially malignant oral mucosal disorders. J Oral Pathol Med. 2013;42(3):243–9.
121. Wu L, et al. Candidal infection in oral leukoplakia: a clinicopathologic study of 396 patients from eastern China. Ann Diagn Pathol. 2013;17(1):37–40.
122. Chiu CT, et al. Candida invasion and influences in smoking patients with multiple oral leucoplakias--a retrospective study. Mycoses. 2011;54(5):e377–83.
123. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
124. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883–99.
125. Feller L, Altini M, Lemmer J. Inflammation in the context of oral cancer. Oral Oncol. 2013;49(9):887–92.
126. Lu R, et al. Inflammation-related cytokines in oral lichen planus: an overview. J Oral Pathol Med. 2015;44(1):1–14.
127. Gandolfo S, et al. Risk of oral squamous cell carcinoma in 402 patients with oral lichen planus: a follow-up study in an Italian population. Oral Oncol. 2004;40(1):77–83.
128. Lodi G, et al. Interventions for treating oral leukoplakia to prevent oral cancer. Cochrane Database Syst Rev. 2016;7:CD001829.
129. Lodi G, Porter S. Management of potentially malignant disorders: evidence and critique. J Oral Pathol Med. 2008;37(2):63–9.
130. Ribeiro AS, et al. A review of the nonsurgical treatment of oral leukoplakia. Int J Dent. 2010;2010:1–10.
131. Gudas LJ. Retinoids, retinoid-responsive genes, cell differentiation, and cancer. Cell Growth Differ. 1992;3:655–62.
132. Hong WK, et al. Retinoid chemoprevention of aerodigestive cancer: from basic research to the clinic. Clin Cancer Res. 1995;1:677–86.

133. Scher RL, et al. Fenretinide-induced apoptosis of human head and neck squamous carcinoma cell lines. Otolaryngol Head Neck Surg. 1998;118:464–71.
134. Gorsky M, Epstein JB. The effect of retinoids on premalignant oral lesions: focus on topical therapy. Cancer. 2002;95(6):1258–64.
135. Paitelli A, et al. bcl-2 expression and apoptotic bodies in 13-cis-retinoic acid (isotretinoin)-topically treated oral leukoplakia: a pilot study. Oral Oncol. 1999;35:314–20.
136. Shah JP, et al. Effects of retinoids on oral leukoplakia. Am J Surg. 1983;146:466–70.
137. Epstein JB, Gorsky M. Topical application of vitamin A to oral leukoplakia - a clinical case series. Cancer. 1999;96:921–7.
138. Tete S, et al. The role of apoptosis and bcl-2 protein in topical treatment of oral leukoplakia with isotretinoin. Minerva Stomatol. 1999;48:411–8.
139. Epstein JB, et al. Topical bleomycin treatment of oral leukoplakia: a randomized double-blind clinical trial. Head Neck. 1994;16(6):539–44.
140. Epstein JB, et al. Topical bleomycin for the treatment of dysplastic oral leukoplakia. Am Cancer Soc. 1998;83(4):629–34.
141. Chan G, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res. 1999;59(3):991–4.
142. Renkonen J, Wolff H, Paavonen T. Expression of cyclo-oxygenase-2 in human tongue carcinoma and its precursor lesions. Virchows Arch. 2002;440:594–7.
143. Mulshine J, et al. Randomized, double-blind, placebo-controlled phase IIB trial of the cyclooxygenase inhibitor ketorolac as an oral rinse in oropharyngeal leukoplakia. Clin Cancer Res. 2004;10:1565–73.
144. Ulrich C, et al. Management of actinic cheilitis using diclofenac 3% gel: a report of six cases. Br J Dermatol. 2007;156(Suppl 3):43–6.
145. Jepsen A, Winther JE. Mycotic infection in oral leukoplakia. Acta Odontol Scand. 2009;23(3):239–56.
146. Galle F, et al. Candida spp. in oral cancer and oral precancerous lesions. New Microbiol. 2013;36:283–8.
147. Diajil A, et al. Clinical outcome following oral potentially malignant disorder treatment: a 100 patient cohort study. Int J Dent. 2013;2013:809248.
148. Hebbar PB, Pai A, Sujatha D. Mycological and histological associations of Candida in oral mucosal lesions. J Oral Sci. 2013;55(2):157–60.
149. Cawson RA, Lehner T. Chronic hyperplastic candidiasis - candidal leukoplakia. Br J Dermatol. 1968;80(9):9–16.
150. Thomas SM, Grandis JR. The current state of head and neck cancer gene therapy. Hum Gene Ther. 2009;20:1565–75.
151. Koch WM, et al. p53 Mutation and locoregional treatment failure in head and neck squamous cell carcinoma. J Natl Cancer Inst. 1996;88(21):1580–5.
152. Shin DM, et al. Activation of p53 gene expression in premalignant lesions during head and neck tumorigenesis. Cancer Res. 1994;54:321–6.
153. Rudin CM, et al. An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. J Clin Oncol. 2003;21(24):4546–52.
154. Shah AY, Doherty SD, Rosen T. Actinic cheilitis: a treatment review. Int J Dermatol. 2010;49(11):1225–34.
155. Robinson JK. Actinic cheilitis a prospective study comparing four treatment methods. Arch Otolaryngol Head Neck Surg. 1989;115:848–52.
156. Warnock GR, Fuller RP Jr, Pelleu GB Jr. Evaluation of 5-fluorouracil in the treatment of actinic keratosis of the lip. Oral Surg Oral Med Oral Pathol. 1981;52(5):501–5.
157. Smith KJ, et al. Topical 5% imiquimod for the therapy of actinic cheilitis. J Am Acad Dermatol. 2002;47(4):497–501.
158. Flórez Á, Batalla A, de la Torre C. Management of actinic cheilitis using ingenol mebutate gel: a report of seven cases. J Dermatolog Treat. 2017;28(2):149–51.
159. Epstein JB, et al. A survey of the current approaches to diagnosis and management of oral premalignant lesions. J Am Dent Assoc. 2007;138(12):1555–62.

160. Stich HF, et al. Response of oral leukoplakias to the administration of vitamin A. Cancer Lett. 1988;40:93–101.
161. Sankaranarayanan R, et al. Chemoprevention of oral leukoplakia with vitamin A and beta carotene: an assessment. Oral Oncol. 1997;33(4):231–6.
162. Hong WK, et al. 13-Cis-retinoic acid in the treatment of oral leukoplakia. N Engl J Med. 1986;315:1501–5.
163. Kaugars GE, et al. Use of antioxidant supplements in the treatment of human oral leukoplakia. Oral Surg Oral Med Oral Pathol. 1996;81:5–14.
164. Nagao T, et al. Treatment of oral leukoplakia with a low-dose of beta-carotene and vitamin C supplements: a randomized controlled trial. Int J Cancer. 2015;136(7):1708–17.
165. Singh M, et al. Efficacy of oral lycopene in the treatment of oral leukoplakia. Oral Oncol. 2004;40(6):591–6.
166. Clinicaltrials.gov. Aspirin Mouthwash in Treating Patients With Oral Leukoplakia. 2013. Accessed 3 Feb 2017.
167. Clinicaltrials.gov. A Randomized Study of Sulindac in Oral Premalignant Lesions. 2016. Accessed 3 Feb 2017.
168. Clinicaltrials.gov. Metformin Hydrochloride in Preventing Oral Cancer in Patients With an Oral Premalignant Lesion. 2017. Accessed 3 Feb 2017.
169. Clinicaltrials.gov. Rosiglitazone Maleate in Treating Patients With Oral Leukoplakia. 2017. Accessed 3 Feb 2017. Available from: https://clinicaltrials.gov/ct2/show/NCT00369174?term=Rosiglitazone+Maleate+in+Treating+Patients+With+Oral+Leukoplakia&rank=1.
170. Haddad RI, Shin DM. Recent Advances in Head and Neck Cancer. N Engl J Med. 2008;359:1143–54.
171. Specenier P, Vermorken JB. Biologic therapy in head and neck cancer: a road with hurdles. Int Scholar Res Net. 2012;2012:1–15.
172. Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor a and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res. 1993;53:3579–84.
173. Therapeutic Goods Administration. Australian Public assessment report for cetuximab, in Australian Public Assessment Record. Woden, ACT: Commonwealth of Australia; 2013.
174. U.S. Food and Drug Administration. FDA Approves first head & neck cancer treatment in 45 years data shows treatment with erbitux extends survival. Baltimore, MD: USFDA; 2006.
175. Agency EM. Erbitux. Human Medicines 2016. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000558/human_med_000769.jsp&murl=menus/medicines/medicines.jsp&mid=WC0b01ac058001d124.
176. Scully C, Eisen D, Carrozzo M. Management of oral lichen planus. Am J Clin Dermatol. 2000;1(5):287–306.
177. Zhang J, et al. Biologics, an alternative therapeutic approach for oral lichen planus. J Oral Pathol Med. 2011;40(7):521–4.
178. Heffernan MP, et al. A single-center, open-label, prospective pilot study of subcutaneous efalizumab for oral erosive lichen planus. J Drugs Dermatol. 2007;6(3):310–4.
179. Bekke J, Baart J. Six years' experience with cryosurgery in the oral cavity. Int J Oral Surg. 1979;8(4):251–70.
180. Sako K, Marchetta FC, Hayes RL. Cryotherapy of intraoral leukoplakia. Am J Surg. 1972;124:482–4.
181. Kawczyk-Krupka A, et al. Comparison of cryotherapy and photodynamic therapy in treatment of oral leukoplakia. Photodiagnosis Photodyn Ther. 2012;9(2):148–55.
182. Kuwahara RT, Rasberry RD. Cryosurgery acne attachment for actinic cheilitis. Dermatol Surg. 2000;26(9):899.
183. Ben-Bssat M, et al. The CO2 laser in surgery of the tongue. Bt J Plast Surg. 1978;31:155–6.
184. Thomson PJ, Wylie J. Interventional laser surgery: an effective surgical and diagnostic tool in oral precancer management. Int J Oral Maxillofac Surg. 2002;31:145–53.
185. Panwar A, Lindau R, Wieland A. Management for premalignant lesions of the oral cavity. Expert Rev Anticancer Ther. 2014;14(3):349–57.

186. Ishii J, Fujita K, Komori T. Laser surgery as a treatment for oral leukoplakia. Oral Oncol. 2003;39(8):759–69.
187. Frame JW, et al. Use of the carbon dioxide laser in the Management of premalignant lesions of the oral Mucosa. J Laryngol Otol. 2007;98(12):1251–60.
188. Flynn M, White M, Tabah R. Use of carbon dioxide laser for the treatment of premalignant lesions of the oral mucosa. J Surg Oncol. 1988;37:232–4.
189. Roodenburg J, Panders A, Vermey A. Carbon dioxide laser surgery of oral leukoplakia. Oral Surg Oral Med Oral Pathol. 1991;71:670–4.
190. Chandu A, Smith AC. The use of CO2 laser in the treatment of oral white patches: outcomes and factors affecting recurrence. Int J Oral Maxillofac Surg. 2005;34:396–400.
191. Chiesa F, Sala L, Costa L. Excision of oral leukoplakias by CO2 laser on an out-patient basis: a useful procedure for prevention and early detection of oral carcinomas. Tumori. 1986;72:307–12.
192. van der Hem PS, et al. The results of CO2 laser surgery in patients with oral leukoplakia: a 25 year follow up. Oral Oncol. 2005;41(1):31–7.
193. Horch H, Gerlach K, Schaefer H. CO2 laser surgery of oral premalignant lesions. Int J Oral Maxillofac Surg. 1986;15:19–24.
194. White J, et al. Nd: YAG and CO2 laser therapy of oral mucosal lesions. J Clin Laser Med Surg. 1998;16:299–304.
195. Schoelcg ML, et al. Laser management of oral leukoplakias: a follow-up study of 70 patients. Laryngoscope. 1999;109:943–53.
196. Chu FWK, SIlverman S Jr, Dedo HH. CO2 laser treatment of oral leukoplakia. Laryngoscope. 1988;98(2):125–30.
197. Chiesa F, et al. Follow-up of oral leukoplakia after carbon dioxide laser surgery. Arch Otolaryngol Head Neck Surg. 1990;116:177–80.
198. Pinheiro A, Frame J. Surgical management of pre-malignant lesions of the oral cavity with the CO2 laser. Braz Dent J. 1996;7:103–8.
199. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol. 1995;79:321–9.
200. Schwarz F, et al. Cytologic and DNA-cytometric follow-up of oral leukoplakia after CO2- and Er:YAG-laser assisted ablation: a pilot study. Lasers Surg Med. 2005;37(1):29–36.
201. Nammour S, et al. Evaluation of different laser-supported surgical protocols for the treatment of oral leukoplakia: a long-term follow-up. Photomed Laser Surg. 2017;35:629.
202. Zelickson BD, Roenigk RK. Actinic cheilitis. Treatment with the carbon dioxide laser. Cancer. 1990;65(6):1307–11.
203. de Godoy Peres FF, et al. A study of actinic cheilitis treatment by two low-morbidity CO2 laser vaporization one-pass protocols. Lasers Med Sci. 2009;24(3):375–85.
204. Orenstein A, et al. A new modality in the treatment of actinic cheilitis using the Er:YAG laser. J Cosmet Laser Ther. 2007;9(1):23–5.
205. Armenores P, et al. Treatment of actinic cheilitis with the Er:YAG laser. J Am Acad Dermatol. 2010;63(4):642–6.
206. Johnson TM, et al. Carbon dioxide laser treatment of actinic cheilitis. Clinicohistopathologic correlation to determine the optimal depth of destruction. J Am Acad Dermatol. 1992;27(5 Pt 1):737–40.
207. Holmstrup P, et al. Oral premalignant lesions: is a biopsy reliable? J Oral Pathol Med. 2007;36(5):262–6.
208. Gomes CC, et al. Inter- and intra-lesional molecular heterogeneity of oral leukoplakia. Oral Oncol. 2015;51(2):178–81.
209. Holmstrup P, Dabelsteen E. Oral leukoplakia-to treat or not to treat. Oral Dis. 2016;22(6):494–7.
210. Arduino PG, et al. Outcome of oral dysplasia: a retrospective hospital-based study of 207 patients with a long follow-up. J Oral Pathol Med. 2009;38:540–4.
211. Saito T, et al. Development of squamous cell carcinoma from pre-existent oral leukoplakia: with respect to treatment modality. Int J Oral Maxillofac Surg. 2001;30(1):49–53.

212. Thomson PJ. Field change and oral cancer: new evidence for widespread carcinogenesis? Int J Oral Maxillofac Surg. 2002;31:262–6.
213. Farah CS, et al. Improved surgical margin definition by narrow band imaging for resection of oral squamous cell carcinoma: a prospective gene expression profiling study. Head Neck. 2016;38(6):832–9.
214. Poh CF, et al. Fluorescence visualization–guided surgery for early-stage oral cancer. JAMA Otolaryngol Head Neck Surg. 2016;142(3):209–16.
215. Farah CS, et al. Efficacy of tissue autofluorescence imaging (velscope) in the visualization of oral mucosal lesions. Head Neck. 2012;34(6):856–62.
216. Menta Simonsen Nico M, Rivitti EA, Lourenço SV. Actinic cheilitis: histologic study of the entire vermilion and comparison with previous biopsy. J Cutan Pathol. 2007;34(4):309–14.
217. Satorres Nieto M, Gargallo Albiol J, Gay Escoda C. Surgical management of actinic cheilitis. Med Oral. 2001;6(3):205–17.
218. Barry RB, et al. Direct primary closure without undermining in the repair of vermilionectomy defects of the lower lip. Br J Dermatol. 2012;167(5):1092–7.
219. Sand M, Altmeyer P, Bechara FG. Mucosal advancement flap versus primary closure after vermilionectomy of the lower lip. Dermatol Surg. 2010;36(12):1987–92.
220. Dougherty T, Schwartz S. Photodynamic therapy for cancer. Nat Rev Cancer. 2003;3(5):380–7.
221. Vohra F, et al. Efficacy of photodynamic therapy in the management of oral premalignant lesions. A systematic review. Photodiagnosis Photodyn Ther. 2015;12(1):150–9.
222. Jerjes W, et al. Photodynamic therapy outcome for oral dysplasia. Lasers Surg Med. 2011;43(3):192–9.
223. Jerjes W, Hamdoon Z, Hopper C. Photodynamic therapy in the management of potentially malignant and malignant oral disorders. Head Neck Oncol. 2012;4:1–7.
224. Clinicaltrials.gov. Photodynamic therapy for oral precursor lesions (PDT). 2016. Accessed 3 Feb 2017.
225. Ozog DM, et al. Photodynamic therapy: a clinical consensus guide. Dermatol Surg. 2016;42(7):2016.
226. Sotiriou E, et al. Actinic cheilitis treated with one cycle of 5-aminolaevulinic acid-based photodynamic therapy: report of 10 cases. Br J Dermatol. 2008;159(1):261–2.
227. Sotiriou E, et al. Photodynamic therapy with 5-aminolevulinic acid in actinic cheilitis: an 18-month clinical and histological follow-up. J Eur Acad Dermatol Venereol. 2010;24(8):916–20.
228. Berking C, et al. The efficacy of photodynamic therapy in actinic cheilitis of the lower lip: a prospective study of 15 patients. Dermatol Surg. 2007;33(7):825–30.
229. Suárez-Pérez JA, et al. Treatment of actinic cheilitis with methyl aminolevulinate photodynamic therapy and light fractionation: a prospective study of 10 patients. Eur J Dermatol. 2015;25(6):623–4.
230. Choi SH, Kim KH, Song KH. Efficacy of ablative fractional laser-assisted photodynamic therapy for the treatment of actinic cheilitis: 12-month follow-up results of a prospective, randomized, comparative trial. Br J Dermatol. 2015;173(1):184–91.
231. Kim SK, Song HS, Kim YC. Topical photodynamic therapy may not be effective for actinic cheilitis despite repeated treatments. Eur J Dermatol. 2013;23(6):917–8.
232. Hsue S, et al. Malignant transformation in 1458 patients with potentially malignant oral mucosal disorders: a follow-up study based in a Taiwanese hospital. J Oral Pathol Med. 2007;36:25–9.
233. Kanatas AN, et al. The configuration of clinics and the use of biopsy and photography in oral premalignancy: a survey of consultants of the British Association of Oral and Maxillofacial Surgeons. Br J Oral Maxillofac Surg. 2011;49(2):99–105.
234. Licitra L, et al. Evaluation of the benefit and use of multidisciplinary teams in the treatment of head and neck cancer. Oral Oncol. 2016;59:73–9.