CHAPTER 20

Screening and prevention of oral cancer

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SUMMARY BOX

- Oral cancer is the sixth most common cancer in the world, with high incidence in countries with a high rate of tobacco usage, especially smokeless tobacco.
- Tobacco, alcohol, areca nut, and human papilloma virus are the most important causative factors in the development of oral cancer.
- Identification of high-risk cases based on tobacco habits and premalignant oral lesions, their screening and effective management, is the most cost-effective means of primary and secondary prevention of oral cancer.
- Primary care physicians, health workers, and mouth self-examination play a very important part in screening of the oral cavity.
- A variety of screening methods are available, ranging from older methods of exfoliative cytology and Lugol's iodine to newer methods using nanoparticles, narrow-band imaging, and lab-on-a-chip.
- Numerous drugs, natural agents, and nutritional supplements have been studied for their role in chemoprevention, but there is insufficient evidence to recommend their use.

Oral cancers are almost always squamous carcinomas [1] and refer to cancers of the oral or anterior two-thirds tongue, gingivo-buccal complex (GBC), floor of mouth, hard palate, and lips. Oral cancer is the sixth most common cancer in the world, with more than 300 000 new cases and 145 000 deaths annually [2]. It is more common in developing countries where tobacco chewing is very prevalent, and is the most common cancer in males in South Asia and parts of South-East Asia. Its incidence is also increasing in Germany, Denmark, Scotland, and central and eastern France. In the Indian subcontinent and some other counties where tobacco chewing is very common, the predominant site of cancer in the oral cavity is the gingivo-buccal complex (GBC). In Europe, the USA, and other regions

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where tobacco abuse is primarily in the form of cigarette smoking with or without alcohol, the tongue is the most common site of oral cancer.

The estimated five-year survival of oral cancer patients is about 81% for localized, 42% for regional, and 17% for distant metastatic disease [3]. The mortality rate is higher in developing countries due to late-stage presentation and limited access to multidisciplinary treatment [1]. Oral squamous cell carcinomas (OSCCs) are generally preceded by premalignant lesions. These premalignant lesions may be very distinct, like the white patch of leukoplakia, the red patch of erythroplakia, and oral submucous fibrosis (OSMF). The long latent period of malignant transformation, the reversible nature of some premalignant lesions, and the high cure rates of early-stage oral cancer are the basis for primary and secondary prevention [4].

Screening

Screening is the process of evaluating an asymptomatic person to determine if he or she is 'likely' or 'unlikely' to have a potentially malignant or malignant lesion [5]. The oral cavity being easily visible and accessible, it is amenable to screening by self-examination, or examination by doctors or health workers [6]. Oral cancers are ideally suited for screening. A cluster randomized trial done in Kerala, India involving 191 873 individuals showed 34% reduction in oral cancer mortality in high-risk individuals with tobacco use and/or alcohol consumption after three rounds of screening and nine years of follow-up [6]. After four rounds of screening and fifteen years of follow-up there was a 38% reduction in incidence and an 81% decrease in oral cancer mortality, which was statistically significant [7]. Targeted screening of individuals using tobacco with or without alcohol is the most cost-effective way. A Cochrane review in 2013 recommends oral cancer screening of high-risk individuals, which will lead to improvement of survival and stage shift across the whole population [8]. The American Dental Association recommends that visual and tactile screening in high-risk individuals may result in detection of cancer in the early stages. There is insufficient evidence to show the benefit of screening asymptomatic individuals without habits [5]. Recommendations regarding screening for oral cancer by various agencies are summarized in Table 20.1.

Role of primary care clinicians and health workers in screening

Primary care clinicians play a very important role in screening high-risk cases who are either tobacco users or have oral premalignant lesions. The sensitivity of clinical oral examination varies from 50% to 99% and specificity from 90% to 98% [9]. Visual examination is the gold standard for detecting early epithelial changes.

Table 20.1 Guidelines for oral cancer screening.

Agency	Recommendations
American Cancer Society [9]	Oral examination should be part of routine health check-ups after the age of 20. High-risk individuals should be screened annually.
American Dental Association [5]	Screening is recommended in high-risk individuals. No benefit of screening in asymptomatic individuals.
U.S. Preventive Services Task Force [10]	Evidence is insufficient to support screening in asymptomatic individuals.
UK National Health Service [11]	Screening programmes among high-risk individuals is the most cost-effective option for screening Screening not recommended in asymptomatic individuals.
Canadian Task Force [12]	Insufficient evidence for or against screening for oral cancer. Annual examination for high-risk individuals.

After an accurate history regarding high-risk behaviour such as use of tobacco and alcohol, a systematic visual and tactile oral cavity examination should be performed. A systematic oral examination can be performed within two minutes with a very basic set-up and can be done equally well in the field or community setting. It is important to evaluate and document orodental health and any premalignant lesion such as submucous fibrosis, leukoplakia, erythroplakia, and so on, and to biopsy if indicated. Health workers should take relevant history and perform oral examination for any restriction in mouth opening or any mucosal changes in the form of ulceration, bleeding, or changes in colour, texture, or mobility [13]. Cases with any suspicious findings should be referred to the clinician for re-evaluation and biopsy if warranted.

Leukoplakic lesions have a higher probability of malignant transformation if they are present in females, occur in the absence of addiction, are larger (>200 mm^2), or in specific sites such as the ventral surface of the tongue, soft palate, and retro-molar trigone [4].

Role of mouth self-examination.

Mouth self-examination (MSE) is a simple but systematic self-examination method performed using a mirror under good illumination. The quality and utility of MSE will depend on the information and training provided by the clinicians or health workers and the use of teaching aids. During MSE, a person should look for any suspicious lesions like white or red patches, ulcers, bleeding, restricted mouth opening, or burning, and report to physicians or screening clinics if any suspicious features are noted. A demonstration project for MSE was conducted in

Kerala, India involving 22 000 individuals. Compliance with MSE was observed in 36% participants. Of the 247 individuals who reported to clinics, 34% had oral precancerous lesions and 3% were diagnosed with cancer [14]. Another study from Kerala evaluated the role of MSE in 57 704 high-risk individuals [15]. Concordance between the findings of MSE and health workers was 77%, and between health workers and clinicians it was 100%. The sensitivity and specificity of MSE were 18% and 99.9%, respectively. MSE detected 42.9% of nonhealing ulcers, 66.7% of red patches, but only 12.7% of white patches. It created over 80% awareness about oral cancer and its risk factors, but the 32% compliance rate was poor and similar to a previous study [14]. A recent Cochrane review [9] shows that the sensitivity of MSE is low (18–33%), but its specificity is high in some studies (54–100%). Considering the lack of better methods which can be applied widely, MSE is recommended for individuals with high-risk behaviour.

Tests for screening (see Table 20.2)

Exfoliative cytology

Exfoliative cytology is the study and interpretation of epithelial cells from the oral mucosa that flake off naturally or artificially with the help of a brush or spatula. It helps in guiding the sites for scalpel biopsy and in monitoring the different regions within a larger lesion [16]. A multicentre study has shown that in 5% of the clinically benign lesions, malignancy was found after biopsy was taken from areas with abnormal exfoliative cytology [17, 18]. The OralCDx® brush biopsy is an oral transepithelial 'biopsy' system using computer-assisted brushing, and helps in identifying dysplasia in areas which are clinically not suspicious [16, 17]. Although

Table 20.2 Sensitivity and specificity of various screening tests for oral cancer.

Test	Sensitivity (%)	Specificity (%)
Exfoliative cytology	52–100	29–100
Toulidine blue	78–100	31–100
Lugol's iodine	87.5	84.2
Chemiluminescence	77	70
Microendoscopy	98	92
Elastic scattering spectroscopy	72	75
Optical coherence tomography	80–98	80–98
Narrow-band imaging	87–96	94–98
Salivary biomarkers	71	75

it has high false-positive rates and overestimates dysplastic lesions, the specimen obtained can be used for further evaluation, including tumour markers and molecular and DNA analysis [16].

Toluidine blue

This is a vital dye which is used to stain abnormal cells and nucleic acids. Surgeons have used it to demarcate epithelial lesions before excision. There is some evidence that toluidine blue (TB) can stain premalignant lesions. Various studies have reported the sensitivity of this technique as from 78% to 100% and specificity from 31% to 100% [16]. Its main disadvantage is that while it stains all areas with malignancy, only 40–70% of dysplastic areas are stained. Gray et al. [19] have shown that toluidine blue is not suitable as a primary method for screening due to the high false-positive rate and low rate in detecting dysplasia [20]. Toluidine blue staining of lesions has been correlated with loss of hetrozygosity (LOH) at 3p, 17p for carcinoma, and with LOH at 9p for dysplasia [21, 22].

Lugol's iodine

Iodine in solution reacts with the cytoplasmic glycogen to produce a colour change. The degree of colour change is directly proportional to the glycogen content of the cell, which is inversely proportional to the keratinization. On staining with Lugol's iodine, the normal cells take a brown or mahogany colour and the dysplastic mucosa does not stain and appears pale. Petruzzi et al. [23] have reviewed the use of Lugol's iodine in oral cancer diagnosis. Almost all studies with Lugol's iodine in oral potentially malignant lesions (OPMLs) or cancers have been done in tertiary centres. It is useful in mapping the suspicious areas for biopsy and to determine the extent of the margin of resection. The sensitivity of this technique is 87.5% and its specificity is 84.2%. There are little data on the utility of this method in a healthy population or in the community setting. Lugol's iodine staining cannot detect subepithelial spread or invasion, and its use is limited to nonkeratinized lesions.

Light-based detection systems

Tissue reflectance was previously used as a method to differentiate between malignant and benign lesions of the cervix. This technique is now also available for similar use in oral lesions [20]. It is based on the principle that the metabolic changes that occur during carcinogenesis cause distinct changes in absorption and refraction on exposure to different types of light [16].

Chemiluminescence

Vizilite® is a well-known system where the subject rinses the mouth with acetic acid and the oral cavity is illuminated with a chemiluminent light stick [17]. It has sensitivity of 100%, but specificity of only 0–14.2%, with a low positive predictive value. To improve its specificity, a combination of this technique with toluidine blue (Vizilite® plus) has been proposed. However, robust scientific evidence to

support its use is lacking [16, 24]. Microlux/DL® is another method using an autoclavable, battery-powered light-emitting diode with a light guide, which produces a diffuse light. The sensitivity and specificity of this technique are 77% and 70%, respectively [16].

Tissue fluorescence imaging

The VELscope® system (Visually Enhanced Lesion Scope), by applying direct fluorescence (400–430 nm), detects the loss of fluorescence in malignant and premalignant lesions [16]. The normal mucosa appears pale green, but the abnormal mucosa appears dark in colour [20]. The sensitivity of this system is 97–100% and specificity is 94–100%. It is helpful in deciding the margins of resection, but its efficacy has not been proven when used in a low-risk population [16]. The IDENTAFI 3000 system is a combination of confocal microscopy, fibreoptics, and fluorescence. It is more accessible than VELscope as it is smaller. It has sensitivity and specificity of 82% and 87%, respectively, in differentiating malignant and nonmalignant lesions [21, 25].

Tissue fluorescence spectroscopy

This consists of an optical fibre that produces excitations of different wavelengths. The data are received by a spectroscope, recorded on a computer, and analysed by special software, thereby reducing subjective interpretation of the results. It is useful in differentiating malignant and nonmalignant lesions, but cannot differentiate between types of lesions, as the optical fibre can sample only a small area at a time [16, 26].

Microendoscopy

This technique is useful for in vivo histopathological assessment. It has a camera attached to a microendoscope with 150X magnification. On comparison with frozen and paraffin section, it has sensitivity and specificity of 98% and 92%, respectively [27, 28].

Elastic scattering spectroscopy

This system generates a wavelength which shows properties within the tissues, like proteins, lipids, nucleus, and other organelles. It has sensitivity of 72% and specificity of 75% in differentiating malignant and nonmalignant lesions. It can also be used to assess the depth of invasion in the mandible and to differentiate types of skin lesions [27].

Optical coherence tomography

Optical coherence tomography uses infrared and records reflectance from the tissue to reconstruct the micro-anatomical architecture. Studies have shown that it co-relates with the histopathological findings, with sensitivity and specificity ranging between 80% and 98%. It can be used to identify skin lesions and to estimate the margin of resection in basal cell carcinoma (BCC) [27].

Narrow-band imaging

This technique uses a narrow-band spectrum filter to identify superficial capillaries and neoangiogenesis in abnormal tissues by enhanced contrast. Abnormal vessels appear as brown dots and dilatations. It is helpful in identifying premalignant and malignant lesions. Various studies report its sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in the range of 87–96%, 94–98%, 73–96%, 97–98%, and 92–97%, respectively [27, 29]. This technique can help in assessment of the primary or recurrent tumour and margins [29]. Narrow-band imaging is not widely used, however, due to the learning curve and lack of robust evidence of its clinical utility [27, 29].

Biopsy

While biopsy is the gold standard, it is an invasive procedure with associated discomfort and also some interpretative issues. For extensive lesions the biopsy should be taken from the most representative area. Incisional biopsy should be of sufficient depth and size, and include the area of the advancing margin of the tumour. Some studies have shown the advantage of punch biopsy over scalpel biopsy in terms of fewer artefacts [30]. Several others have described high inter- and intra-observer variability in diagnosing dysplasia, with concordance of only 56%, and limited reproducibility could lead to more aggressive treatment [17, 30, 31].

Biomarkers, microscopy, and other methods

Various biomarkers, microscopy, and other techniques have been developed and evaluated for diagnosing or characterizing oral premalignant lesions and cancers. A general description is provided here, but the findings of specific studies are beyond the scope of this chapter.

Salivary biomarkers

Several studies have evaluated the role of salivary biomarkers for screening and diagnosis of OPML and oral cancer. Most commonly studied salivary markers are Cyfra 21-1, CA 19-9, CA 125, carcinoembryonic antigen (CEA), and tissue polypeptide-specific antigen (TPS). The presence of Cystain SA-I deletion in saliva is a marker of oral tumours. The sensitivity, specificity, negative, and positive predictive value of significant increase (400%) in salivary concentrations of Cyfra 21-1, TPS, and CA 125 are 71%, 75%, 71%, and 75%, respectively [17]. Levels of IL-8 and Il-1β are also elevated in oral cancer patients [32].

Serum biomarkers

Increased levels of plasma micro RNA mir-31 and circulatory vascular endothelial growth factor (VEGF) in serum are markers of OSCC. Autoantibody against p53 is detected in 25% of patients with head and neck squamous cell carcinoma (HNSCC). OSCC is associated with increased levels of C16 and C24 and a decrease in C18-ceramide [32].

DNA analysis

DNA image cytometry determines the malignant potential of cells by measuring ploidy status. Combined cytogenetic and morphological analysis of oral brush samples is helpful in detecting premalignant cells [17]. Samples are assessed for loss of heterozygosity at 3p14 and 9p21, p53 protein expression, chromosomal polysomy, and so on [4]. In a study with 25 patients, allelic imbalance was present in 40% of patients with leukoplakia as compared to controls, giving it sensitivity and positive predictive value of 78% and 100%, respectively [17, 33].

Spectral cytopathology

In this technique, the biochemical composition of exfoliated cells is evaluated by infrared micro-spectral measurement, which is followed by multivariate analysis. The spectral patterns produced are specific to the disease [17].

Multispectral digital microscope

This takes images in narrow-band reflectance, fluorescence, and orthogonal polarized reflectance modes. It helps in differentiation between malignant and nonmalignant lesions [17].

Spectroscopy

Raman spectroscopy, named after its discoverer, Sir C.V. Raman, is a biophysical technique based on inelastic scattering of light emitted from a diode laser. It is able to identify between normal mucosa, premalignant lesions, and malignant lesions [34].

Lab-on-a-chip

This represents the miniaturization of lab analytical procedures on a single chip. It involves microfluidics, which is the chemistry equivalent of silicon chips. The lab-on-a-chip analyses cell membrane proteins that are uniquely expressed on malignant or dysplastic cells [20].

Nano-particles

This is an emerging technology where gold nano-particles are conjugated to anti- epidermal growth factor receptor (EGFR) antibodies. These contrast agents have the potential to extend the ability of vital reflectance microscopies for in vivo molecular imaging. They can potentially enable combined screening and detection [35].

Prevention of oral cancer

Tobacco, alcohol, and betel quid are the most significant risk factors for oral cancer. Countries in the Indian subcontinent, Taiwan, and some South-East Asian and African countries have a high incidence of oral cancer due to the widespread use of smokeless tobacco. In these regions, the most common site of oral cancer is

gingivo-buccal, followed by the oral tongue. Prevention is the most cost-effective strategy for long-term control of cancer. A national cancer programme can help policymakers make the best use of available resources for the benefit of the population [1]. Awareness, screening programmes, and enabling legislation for tobacco control and early detection of OPML and oral cancer form the best way of preventing oral cancer morbidity and mortality. The precancerous changes are reversed after cessation over a period of time. Important known carcinogens for oral cancer and their associated risks are described here.

Smokeless tobacco, betel quid, and areca nut

Smokeless tobacco and preparation of areca nut are available in various combinations and forms around the world. Areca nut and betel quid are especially prevalent in the Indian subcontinent, parts of South-East Asia, Taiwan, and North and East Africa. In the Indian subcontinent, traditionally the areca nut was eaten in betel leaves prepared with slaked lime and acacia Catechu, with or without tobacco. Lime lowers the pH, thereby increasing the absorption of nicotine. In the last few decades, processed betel quid with areca nut has become widely available as ready-to-eat pouches. These may contain tobacco (Gutka) or may not contain tobacco (Pan Masala). Other forms of betel quid with tobacco are zarda and mawa. According to the Global Adult Tobacco Survey (GATS) 2009, 25.9% of adults in India use smokeless tobacco [36]. Smokeless tobacco has 28 known carcinogens, which include tobacco-specific nitrosamines (most harmful), N-nitosamino acids, benzopyrenes, volatile N-nitrosamines, nickel, cadmium, and arsenic [37].

A recent meta-analysis and systematic review of 21 case-control studies and 4 cohort studies confirms that smokeless tobacco and betel quid without tobacco are independent risk factors for oral cancers [38]. For smokeless tobacco, in the 14 case-control studies (4553 cases, 8632 controls) under the random effects model, the adjusted main effect summary computed was an odds ratio (OR) of 7.46 (95% confidence interval [CI] 5.86–9.50, $p=0.001$). The adjusted main effect summary for 4 cohort studies (163 430) was a relative risk (RR) of 5.48 (95% CI 2.56–11.71, $p=0.001$). For betel quid without tobacco, in the 15 case-control studies (4648 cases; 7847 controls) under the random effects model, the adjusted main effect summary computed was OR 2.82 (95% CI 2.35–3.40, $p=0.001$). The risk conferred by betel quid without tobacco is presumably due to the areca nut.

Tobacco smoking

Cigarettes are the most common form of smoked tobacco around the world. A meta-analysis reported a pooled RR of 3.43 (95% CI 2.37–4.94) for oral cancer in 12 studies comparing current smokers with never smokers, and a RR of 1.4 (95% CI.99–2.0) in 9 studies comparing former smokers with never smokers [39]. Most studies included in this meta-analysis are from Europe or North America where the predominant form of tobacco use is cigarette smoking. In the Indian subcontinent use of bidi is very common. Bidis are made of sun-dried flaked tobacco rolled in dried tendu or temburni leaf [40]. The cancer risk associated with bidi

was examined in a large cohort of 66 277 men in the age range of 30–80 years in Kerala [40]. This study reported that tobacco chewing was associated with increased oral cancer risk ($p<0.001$), and that the risk increased with greater frequencies ($p<0.001$) and duration ($p<0.001$) of use. While an increased risk of oral cancer with alcohol was not found in this study, bidi smoking in men without tobacco chewing was associated with an increased oral cancer risk, with RR 2.6 (95% CI 1.4–4.9). This risk increased with duration and frequency of bidi smoking ($p<0.001$) and younger age at start of bidi smoking ($p=0.007$). After smoking cessation, the level of risk for cancer approaches that of a nonsmoker in about 10 years [41].

Alcohol

Alcohol is an important risk factor for oral cancer, as it may potentiate the carcinogenic effects of tobacco with a multiplicative rather than additive effect [42]. A meta-analysis [43] reported a slight increased risk for oral cancer in light drinkers (RR 1.17, 95% CI 1.01–1.35) and a higher risk in heavy drinkers (four or more drinks; RR 4.64, 95% CI 3.78–5.70). Alcohol cessation leads to a 40% decrease in the risk of oral cancer after 20 years or more of cessation as compared to drinkers [41].

Human papilloma virus

The human papilloma virus (HPV) is an important risk factor for oropharyngeal cancers. Association of HPV has been seen in other head and neck cancer sites, and one study reported HPV in 25% of oral cancers, with the majority being HPV-16 followed by HPV-18 [44].

Diet and nutritional factors

In developing countries 20% of cancers are related to dietary factors. A diet rich in fresh fruits and vegetables is protective against oral cancerous and precancerous lesions [1]. Foods protecting against oral cancer include fish, pulses, and buttermilk. Foods protecting against oral precancer are fibres, tomatoes, and those containing vitamin C, iron, copper, zinc, and other micronutrients [41]. Consuming very hot liquids increases the risk of oral cancer [1].

Genetic predisposition

While environmental factors play a major role in the development of oral cancer, a weak inherited susceptibility for oral cancer may be conferred due to specific gene–environment interactions. A family history of HNSCC has an RR of 3.5–3.8 for first-degree relatives. Null genotype or mutation in genes coding for detoxifying enzymes, glutathione S-transferase (GST), and UDP-glucuronyl transferase 1A7 (UGT1A7) are associated with an increased risk of oral cancer. Germline p16 and p53 mutations also predispose to an increased risk for oral cancer. Various cancer syndromes, like Bloom's syndrome, Fanconi's anaemia, and ataxia telengectasia, have a defective DNA repair mechanism and are associated with an increased risk of oral cancer [45]. A study through whole exome sequencing has

identified several recurrent and novel somatic genetic alterations in gingivo-buccal cancer, which is the most common type of oral cancer in the Indian subcontinent [44].

Chemoprevention of oral cancer

Chemoprevention is the use of drugs or natural products that can reverse or arrest malignant transformation. Foy et al. [4] have reviewed the role of chemoprevention in oral premalignant lesions. Use of retinoids in a secondary setting produces a clinical and histological response in 64% and 57% cases of OPML, respectively, but may cause hypertriglyceridemia, mucocutaneous effects, and relapse after stoppage. Use of retinoids with N-acetylcysteine or COX-2 inhibitors has not shown any benefit so far. Thiazolidinediones have shown promising results in a Phase IIa study, with a 68% histological response in leukoplakia, and further Phase IIb studies are under way. Numerous dietary agents like green tea polyphenols, curcumin, herbs, fruits, and vegetables have been reported to show promising chemopreventive effects. Curcumin from turmeric has been shown to downregulate nicotine-induced COX-2 and nuclear factor-KappaB (NF-kB) in vitro. Spirulina, black raspberries, vitamin supplements, resveratrol, and lycopene have all been evaluated for their protective role against OPML and OSCC. Attempts at restoring p53 function using a genetically engineered virus have been made using ONYX-015, but the response is short-lived and unpredictable [4]. Erlotinib, an EGFR inhibitor, is being evaluated in a Phase III trial as an oral chemopreventive agent for OPML with LOH. However, for none of these agents is there high-quality scientific evidence of clinical benefit to recommend their use outside clinical trials.

Conclusion

Oral cancer is ideally suited for prevention and early detection, but it continues to be a major health problem in many parts of the world. Screening of high-risk individuals using simple methods is a proven and cost-effective way of reducing morbidity and mortality from oral cancer. Mouth self-examination and physicians have very important roles to play in screening and effective management of this disease. There are various tests available for screening, but a test with high sensitivity, specificity, and cost-effectiveness is still not available. Almost all cases of oral cancer can be prevented by abstaining from tobacco and alcohol. Several natural agents and anti-oxidants have shown promise as chemopreventive agents, and their role in oral cancer prevention needs to be established through large randomized trials with suitable surrogate biomarker end-points. Health campaigns are required to create public health awareness about mouth self-examination, the risks of various forms of tobacco use, the effective management of

precancerous lesions, and tobacco cessation programmes for high-risk cases. Governments need to devise strong strategies and legislation for reducing the production, marketing, and use of tobacco products.

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