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A REVIEW ON CURRENT SCENARIO OF ORAL CANCER IN INDIA WITH SPECIAL EMPHASIS ON MODERN DETECTION SYSTEMS AND BIOMARKERS

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ABSTRACT

Oral squamous cell carcinoma is a major public health concern worldwide and a growing threat for rapidly developing economies such as India, where it ranks among the top three cancers. This review aims to discuss the national status of oral cancer in terms of incidences and mortality. We have added the emphasis on clinical characteristics of oral potentially malignant disorders and emerging optical diagnostic techniques to detect oral lesions which would otherwise go undetected by a conventional oral examination. Modern detection systems such as autofluorescence, chemiluminiscence, Narrowband imaging and Raman spectroscopy will definitely aid Conventional oral examination for diagnosis. Definitive diagnosis of oral cancer by using saliva and serum-based noninvasive biomarkers can minimize the need of tissue biopsies and patient discomfort. Urgent research efforts are required to find new ways to identify and examine high-risk population for the early diagnosis and prevention of Oral squamous cell carcinoma.

Keywords: Biomarkers, Detection Methods, Early Diagnosis, Oral Squamous Cell Carcinoma

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INTRODUCTION

Ever-increasing burden of cancer becomes a major public health concern worldwide, posing a high impact on world health. Mortality due to cancer now exceeds the deaths caused by coronary heart diseases or all strokes [1]. International Agency of Research on Cancer (IARC) estimated 14.1 million new cancer incidences and 8.2 million cancer deaths worldwide in its online database GLOBOCAN 2012. Constant growth has been observed in cancer incidence and mortality, which were 12.7 million and 7.6 million, respectively in 2008. Prevalence data indicated that 32.6 million people are living with cancer diagnosed in the previous five years. Estimated cancer incidences are expected to raise up to 20 millions in 2025 [2]. Oral cancer (International classification of diseases, 10th edition, ICD-10 codes: C00–C08) ranks 15th globally with high prevalence and mortality in less developed countries. About 90% of oral cancers are oral squamous cell carcinoma (OSCC) [3, 4]. OSCC includes anatomical sites such as lip mucosa, dorsal, ventral, and border of the anterior two-third of the tongue, vestibule, gums, hard palate, chick mucosa and retromolar area. It shows poor prognosis and consistent treatment effort have not improved the 5 y survival rate, which is about 50-63% [5]. Additionally, oral cancer treatment causes disfigurement many times, affecting the patient's quality of life permanently. Cancers of the lip and oral cavity accounted for about 300,000 incidences (2.1% of world total) and 145,000 (1.8% of world total) deaths worldwide. Two-third oral cancer loads have been found in males. 77% of the total oral cancer incidences have been observed in less developed regions. Non-communicable diseases causes 50% deaths in India among which 6% are attributed to cancer and are expected to rise in the near future due to population growth. More than 1 million cancer cases are diagnosed each year and projected to raise upto 1.7 million in 2035. More than 70% deaths due to cancer occurred in productive age of individuals (30-69years). Age standardized incidence rate for all cancers is 94 per 100,000 individuals [6, 7]. Incidence of OSCC in India is higher compared to the western world due to regional variations and pattern of habits. Tobacco is considered to be main causative factor for most premalignant and malignant lesions of the oral cavity. Unique indigenous tobacco preparations along with areca/betel nut (Gutakha, Mawa etc.), overconsumption of alcohol, poor diets, poor oral hygine and persistent infections of the upper aerodigestive tract with human papilloma virus (HPV) significantly increases oral cancer risk [8]. Consumption of tobacco and betel quid with synergistic alcohol effects are unique to India. Late appearance of clinical signs and symptoms of OSCC carries significant morbidity and mortality. A recent systematic review by Omar [9] concluded saliva-based oral diagnosis and optical diagnosis are promising noninvasive diagnostic modalities for the early detection of OSCC. Saliva based biomarkers can help to monitor the disease status of dysplasia between biopsies. Salivary diagnostics still requires validation [10]. While a review by Liu *et al.*, summarizes although non-invasive techniques show potential for detecting OPMD standalone use of these techniques cannot be recommended in clinics [11]. Large sample size prospective studies are needed for screening with these tools. Similar conclusions are drawn by Iyer *et al.* [12] where they find insufficient evidence for the use of adjunctive optical screening techniques in routine clinical practice but salivary biomarkers can be a potential screening tool. Regular visual oral screening of high risk population also benefit in reducing mortality in geographic areas where OSCC is more common.

Here, we review published data on the national status of oral cancer in terms of incidences, age-adjusted incidence rates (AAR), mortality, trends, regional variations and incidences according to sex in India. We have also briefly discussed the major risk factors for the development of OSCC with its possible implications and mode of action. We have also added the emphasis on clinical characteristics and risk factors for the development of oral potentially malignant disorders (OPMD). We also tried to explore the emerging optical diagnostic techniques to detect oral lesions that would otherwise go undetected by conventional oral examination. Definitive diagnosis of oral cancer by using saliva and serum-based non-invasive biomarkers will minimize the need of tissue biopsies and patient discomfort.

Current status of oral cancer in India

Oral cancer is major cause of mortality and morbidity in India. It is the third common cancer type next to breast and cervix cancer, which accounts for about 30% of the total cancer cases in the country. India share quarter of the world burden of oral cancer.

Around 77,000 new cases diagnosed and 52,000 people die each year due to oral cancer [13]. Cancers of Gingio-buccal complex (buccal mucosa, lower alveolus, retromolar trigone) comprises 60% oral cancer cases hence typically called as 'Indian oral cancer' [14]. National cancer registry programme (NCRP) report (NCRP 2012-2014) summarized oral cancer as second and third most common cancer affecting men and women respectively. Oral cancer is the leading cancer type in males of western states cancer registries such as Barshi Rural/expanded, Ahmadabad Urban, Nagpur, Pune and Wardha. It is also the leading cancer type in Bhopal and second leading in Mumbai cancer registries. Ahmadabad cancer registry shows highest age incidence rate of oral cancer about 28.7 per 100000. AAR variable nationwide and is about 20 per 100,000 population, which is again highest in comparison with 10 per 100,000 in the USA, and 2 per 100,000 in the Middle east. Incidences of OSCC are more common in men than women. Increasing trends of incidence rates in men of about 1.4 to 7% has been observed in various PBCR while it remained constant or decreased slightly in women. Mumbai based cancer registry showed 2.7% annual increase in age-standardized incidence rates of oral cancer in last 15 y in men [15]. East Khasi Hills in Meghalaya state shows the highest AAR (9.4) of oral cancer in females. Estimated national mortality of oral cancer is 6.7 per 100,000 in men and 3 per 100,000 in women. A national representative field report Million Death Study (MDS) from 1.1 million homes across the country reported oral cancer (including pharynx) as most common fatal cancer in men (22.9% deaths) while ranks fourth in women (9.8% deaths). According to GLOBOCAN online analysis prediction (Accessed on 29.11.2016) oral cancer cases in India will significantly increase from 77003 in 2012 to 94903 (66025 males and 28878 females) in 2020. Mortality due to oral cancer is also likely increase from 52067 in 2012 to 64245 (44492 males and 19753 females). Urgent efforts are required to educate people about the established risk factors of OSCC. Otherwise, this situation will further get worsen and may transform into oral cancer epidemics in India.

Oral potentially malignant disorders (OPMD)

OSCC is a result of malignant transformation from precancerous lesions caused due to various environmental factors. Precancerous lesions of oral mucosa are altered areas of tissues which have undergone malignant change during follow up. These premalignant phases show some chromosomal, genomic and molecular alterations found in clearly invasive oral cancers [16, 17]. These are termed 'epithelial precursor lesions'. Clinicians are well versed about characteristic clinical presentation and risk of malignant transformation of pre-cancerous lesions. But general population is largely unaware about symptoms and consequences of precancerous lesions. There is an urgent need to educate people to seek treatment in the early stage [18]. Pathologic findings have limitations in predicting risk of malignant transformation of OPMD and specify the need of molecular markers, which can truly predict the risk of malignant transformation.

Leukoplakia

Oral leukoplakia (OLP) is most common premalignant lesion having wide risk of malignant transformation ranges from 0.13 to 34% [19]. Global prevalence rate ranges 0.5 to 3.4%. Smoking is high-risk factor while alcohol, HPV infection, candidiasis and lowered vitamin A and beta carotene are also found to be possible risk factors in the development of OLP [20]. Variable incidence rates have been observed in India according to tobacco chewing practices. Bihar has 0.2%, Andhra Pradesh has 4.9% while Gujarat has 11.7% prevalence [21]. It can be defined as white plagues of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer. It show atrophy or hyperplasia (acanthosis) and may or may not demonstrate epithelial dysplasia and have assessable tendency to malignant transformation. It is found in the oral cavity, lip vermilion, buccal mucosa and gingival. Clinically leukoplakia shows two types as homogenous and non-homogenous. Homogenous leukoplakia shows low risk of malignant transformation, while non-homogenous leukoplakia carries higher risk of malignant transformation. Non-homogenous leukoplakia includes three varieties-Speckled leukoplakia is a mixed white and red lesion but retaining red character. Nodular leukoplakia is a rounded red or white excrescence while verrucous leukoplakia shows wrinkled or corrugated surface appearance. Proliferative verrucous leukoplakia (PVL) is an aggressive form of oral leukoplakia having considerable morbidity and strong potential for malignant transformation [22]. PVL generally afflicts old age population over 60 y. Many cases of PVL are resistant to all forms of treatments like laser microsurgery, surgical excision and radio and chemotherapy. Smokers carry six times more risk of development of oral luekoplakia than non-smokers, while alcohol is also a major risk factor [23].

Erythroplakia

Oral erythroplakia (OE) presents as a bright red velvety plaque, which cannot be defined clinically or pathologically. When this red patch has white areas within or surrounding it is termed as erythroleukoplakia [24]. Incidences of oral erythroleukoplakia are rare (0.2-0.8%) occurs most commonly in middle-aged and older men [25]. Although uncommon (prevalence 0.02-0.2%), OE shows very high malignant potential, which is about 44.9%. Large population based studies are required to obtain actual prevalence. Biopsy examination revealed 91% OE was dysplasia, carcinoma in situ or cancer. Tobacco chewing is the strong risk factor in Indian population (OR-19.8) with a strong dose response relationship for frequency and duration [26]. Histopathological examination of OE is the only method that can be used to evaluate if there is concomitant dysplasia, carcinoma in situ, or carcinoma within erythroplakia, which is also the same for oral leukoplakia. Before a surgical biopsy is conducted, epithelial status is generally not known. It has been shown that endoscopic technique helpful in detecting early cancerous lesion in the upper aerodigestive tract, including esophagus, pharynx, and oral cavity; abnormal vascular architectures of oral mucosa appear as increased number, tortuous, dilated, twisted, elongated, and corkscrew-type small blood vessels of varying caliber [27] showed that twisted elongated and destructive patterns of intraepithelial microvasculature of endoscopic images are crucial indicators for detecting high-grade dysplasia, carcinoma in situ, and invasive carcinoma in OE.

Lichen planus

Lichen planus (LP) is T-cell mediated chronic inflammatory disorder that affect skin and mucous membranes. Oral Lichen Planus (OLP) is an autoimmune disease of unknown etiology in which local recruitment of CD 8 T lymphocytes induce apoptosis of basal epithelial cells. OLP affects 0.5 to 2.6% of general population [28]. In Indian population, incidence rate of OLP is 2.6% with incidence prevalence in females [29]. It carries risk of malignant transformation unlike to that of cutaneous lichen planus. Calculated risk of malignant transformation of OLP is approximately 1.63% after 6 y of initial diagnosis [30]. Reticular, erythematous (atrophic) and erosive (ulcerated, bulbus) are clinical subtypes recognized for OLP. Atrophic and erosive clinical subtypes are characterized by mild burning to intense pain which may interfere swallowing and speaking abilities of the affected individual while reticular subtype lesions are asymptomatic [31]. OLP is commonly seen on the buccal mucosa as well as it manifests the tongue, gingival or mucobuccal fold. Reticular lichen planus (RLP) shows high occurrence followed by erosive form. Stress and various systemic illnesses importantly, hypersensitivity reactions are associated with OLP. It can be diagnosed correctly by clinical manifestations however, clinical diagnosis is confirmed by oral biopsy with histopathology to rule out dysplasia and malignancy.

Oral submucous fibrosis

Muthubabu K [32] defined Oral submucous fibrosis (OSF) is a disease with unknown aetiology and is a legacy of Indian Population. OSF is characterized by abnormal accumulation of collagen fibre in the oral submucosa. Habit of betel nut or areca nut chewing, prolonged irritation due to tobacco and chillies may be causative factors for OSF. Vitamin B deficiency and Iron deficiency has also been noted in patients with OSF [33]. Early forms of OSF are shows clinical symptoms such as burning sensation, vesiculation, blanching of mucosa and leathery mucosa while late forms are

presented with fibrous bands within mucosa, narrowing of oropharyngeal orifice, woody changes to mucosa and tongue and limitation of mouth opening.

Oral squamous cell carcinoma (OSCC)

OSCC accounts for more than 90% of cancers of oral cavity and oropharynx. It is a malignant condition of oral mucosal epithelium occurs at any anatomical site in the oral cavity. It has ability of local, regional and distant spread. Invasive cancers shows clinical symptoms such as persistant ulceration, tissue proliferation or destruction, red and white color varieties, progressive growth of the area affected, pain, and loss of function etc. OSCC shows one or more clinical symptoms of invasive cancers that can be detected by routine examination [34]. Possible triggering factors for OSCC are exposure to tobacco, alcohol and HPV. Role of human papilloma virus (HPV) in carcinogenesis of OSCC seems to be very minimal. Several studies provide circumstantial evidence of a significant epidemiological association between Epstein–Barr virus (EBV) and OSCC. Tongue cancer in young patient without exposure to tobacco, alcohol and HPV has a distinct genetic profile such as mutations of tumour suppressor genes p53 [35]. Annertz K *et al.* [36] shown the increasing incidence of OSCC in young patients is mainly associated with an up to 6-fold increased occurrence of tongue carcinomas.

Detection methods

Despite the fact that oral cavity is easily accessible for routine examination of oral lesions majority of OSCC patients are diagnosed at a late stage and shows recurrence of cancer after initial treatment in those with lymph node metastasis [37]. 5 y survival rate of OSCC patients remained low since past few decades. High mortality is often associated with delayed diagnosis. Five year survival rate is about 80% to 85% at early stage diagnosis and reduced to 50% at late stage presentation [38]. Detection of precancerous lesions in susceptible population can significantly reduce the incidences of OSCC. Traditional methods such as visual conventional oral examination alone or by using aids such as vital stains are still proved to be economical and effective. Sensitive detection techniques for the OSCC and OPMD have been summarized in table 1.

Table 1: Summary of diagnostic methods for OPMD and OSCC

Category	Diagnostic method	Method of analysis
Clinical methods	*Conventional oral examination	Visual examination of oral cavity with incandescent light [35].
	*Vital staining with toluidine blue	Visual examination of oral cavity with incandescent light.
	*Florescence Visualization (VEL scope)	After TB staing for the presence of dark blue (malignant) and pale blue (OPMD) area [36].
	*Chemiliminiscence (Vizilite)	Scanning of oral cavity for the presence of dark areas illuminated with 400-460 nm wavelength light [37].
	*Narrow Band Imaging	Scanning of oral cavity for the presence of aceto-white areas [38].
	*Raman Spectroscopy	Computed IPCL pattern analysis of oral lesions. Generation of Raman Spectroscopy subjected to computer analysis [7].
Microscopy	*Histopathology	Microscopic examination of tissue for the assessment of epithelial dysplasia [40].
	*Cytopathology	Microscopic examination of disaggregated cells for the assessment of cellular dysplastic changes [4].

Modern detection techniques are relatively expensive and associated with controversial diagnostic outcomes but still have potential to improve diagnosis of OPMD and OSCC.

Conventional oral examination (COE)

Conventional oral examination with thorough history by healthcare professionals regularly for all patients' especially high-risk individuals is an effective early detection technique. A huge cluster randomized controlled screening trial of high-risk population initiated in 1996 in Kerala, India. Screening group (n=96 517) and the control group (n=95,356) were followed up by oral visual examination for the period of 15 y. Screen positive individuals were referred to the study clinicians. Interim reports [39, 40] published out of this study reported that regular oral visual screening in high-risk individuals can significantly reduce oral cancer mortality. 15 y follow up with 3 to 4 rounds of visual screening in and/or alcohol users reduced 47% incidence mortality by 81% [41]. Visual oral examination with normal light is the effective conventional method at least for some anatomical sites. Oral lesions should be carefully observed for its size, colour, texture and outline. White, red and white, ulcerated and/or indurated lesions needs special attention. Various tools such as Toluidine blue, cytopathology and optical imaging systems are used for the confirmation of suspicious oral lesions [38]. Routine oral examination is proved to be effective means of detection of precancerous lesions and OSCC at early stage as incidences of oral cancer are high in India.

Toluidine blue

Toluidine blue (TB) staining is an easy, inexpensive and non-invasive tool being used traditionally for the detection of premalignant and malignant lesions of the oral cavity. TB is a metachromatic dye that stains acidic components of cells. It binds phosphate bonds and intensity of staining depends on amount of DNA which is again proportional to number of nuclei present in the cells of superficial layers. Dark blue stained lesions are considered to be malignant while premalignant lesions appear pale blue [43]. TB staining test for oral lesion includes 20 second oral pre rinse of 1% acetic acid, 20 second water rinse followed by TB solution rinse. It is then followed by post rinse of 1% acetic acid and water rinse [44]. Inflammatory lesions and healing ulcers may get stained dark blue and produces false positive results. Patient follow up reduces false positive interpretation of such lesions. However, clinical expertise and training also fluctuates the outcomes of the test. But still TB is proved to be an efficient diagnostic aid which helps clinicians for the patient referral to expert health care providers for proper diagnosis and treatment.

Histopathology

Incisional and excisional biopsy followed by staining and microscopic examination by pathologist is the gold standard for the diagnosis of OPMD and OSCC. It allows assessment of severity of epithelial dysplasia as mild, moderate and severe which is an important prognostic indicator. However, removal of biopsy is an invasive procedure. Identification of early neoplastic changes and pre-cancerous lesions are associated with high interobserver variations [45].

Cytopathology

Brush cytology and scalpel biopsy are current diagnostic approaches for oral cancer (FDI world dental federation, 2015). Brush cytology is a less invasive method which enables examination of disaggregated cells of mucosal epithelial tissue under light microscope. Despite its less specificity, it is commonly practiced technique in the clinical setting for patients in which scalpel biopsy is not advisable and for follow up of diagnosed lesions. Oral CDx system is less invasive computer assisted cytomorphometric analysis tool for brush biopsies. Transepithelial cell and tissue samples are collected by using specialized disposable brush and fixed on glass slides. Samples are stained with modified Papanicolaou test (Pap test). Computer based image processor

analyses scattered cells for their abnormal morphology with high precision. Samples with no epithelial abnormality are reported as negative, with abnormal epithelial changes as atypical and with definitive dysplastic changes as positive. Atypical and positive patients have to be reffered to undergo incisional biopsy for definitive diagnosis. However, Oral CDx system is a much expensive cytology technique and attracted controversies by producing false negative results [46].

Light based detection systems/Optical diagnostics

Traditional visual, oral examination limits non-specialists in the field to identify premalignant and malignant oral lesions and may results in false negative/positive and may cause further referral and treatment delay. Modern optical diagnostic systems are currently improving to overcome this limitation [47]. Optical diagnostics systems detect changes in the optical properties of neoplastic tissues in oral cavity caused due to metabolic and structural changes during the process of transformation. Modern optical systems based on autoflurescence, chemiluniscence, narrow band imaging and Raman spectroscopy are gaining clinician's attraction as a method for the diagnosis of oral cancer. Optical diagnostic visualization aids reduce patient discomfort and pain caused due to invasive procedures.

Fluorescence visualization (FV)

Pathological differentiation of premalignant lesions for their potential malignancy is still challenging. It needs clinical and biopsy examination which is an invasive process. Fluorescence guided examination of oral cavity is emerging as a non-invasive tool for identification of potentially malignant and malignant lesions. It is based on the differential scattering of light by cellular components upon excitation with specific wavelength of light. SCC and high-grade dysplasia can be distinguished from normal tissue with high sensitivity with FV but less specific in identifying low-grade dysplasia [48]. Many molecules naturally present in the cells such as nicotinamide adenine dinucleotide (NADH), Flavin adenine dinucleotide (FAD), collagen, tryptophan, elastin, keratin, haemoglobin etc. shows autoflurescence when irradiated with specific wavelength of light. These fluorophores have specific excitation and emission wavelengths. Their altered concentration and change in fluorescence pattern can be detected in potentially malignant lesions. Visually Enhanced Lesion Scope (VEL scope) is WHO endorsed and US FDA approved device used by many dentists worldwide. It emits blue light (400-460 nm wavelength), causing pale green oral cavity auto fluorescence. Normal oral mucosa display pale green florescence while abnormal tissue appears dark as a result of phenomenon called a loss of auto fluorescence (LAF). Clinician can then examine oral cavity effectively for the changed florescence pattern in doubtful lesions [49]. This method is non-invasive, safe and reduces biopsies and use of dyes. A literature study shows the specificity of device ranges from 30% to 100% in detecting malignancy and dysplasia. But controversies have been observed to use VEL scope as a routine diagnostic aid for new cases as it is associated with false positive diagnosis ranging from 15-81% which leads to over referrals and increases patient anxiety. Clinically benign lesions show LAF many times when observed with VEL scope leads to over referrals. LAF is not observed with VEL scope in lip moderate dysplasia and increases chances of false negative diagnosis, which is more worrisome false positive diagnosis with VEL scope can be reduced by keen clinical interpretation and periodic review of patient [50].

Chemiluminescence

Chemiluminescence is described as emission of light from a chemical reaction. Use of chemiluminiscent devices for head and neck regions was approved by US FDA in 2002. This technology is marketed as ViziLite and ViziLite Plus Systems and MicroLuxTM/DL systems. This device is a capsule consists of outer flexible plastic coat having fragile glass vial inside. Bending the capsule would results in a reaction between acetyl salicylic acid and hydrogen peroxide. This reaction produces blue-white chemiluminiscence of 430-580 nm wavelength, which lasts for 10 min. All three methods require oral acetic acid rinse for 1 min. which removes surface glycoproteins and causes cellular dehydration. Normal cells of buccal mucosa absorb blue light while dysplastic cells having high nuclear/cytoplasmic ratios reflect light and appear 'aceto-white' [51]. ViziLite can effectively detect malignant lesions. More correct diagnosis of lateral spread of lesion could be possible with ViziLite than clinical examination alone [52]. However, chemiluminiscence detection method has many limitations. It can effectively detect leukoplakias but failed to detect erythroplakias. False positive and false negative results produced by this method are a matter of worry. It is expensive and needs dark environment for observation. Permanent record cannot be generated except photography. Depth of the lesion cannot be predicted, which is an important determinant of malignancy [53]. Chemiluminiscence detection is less specific for the detection of premalignant lesions and may leads to misdiagnosis in the hands of the general dental practitioner.

Narrow band imaging (NBI)

Abnormal angiogenesis is characteristic feature of all types of tumors. As a result of metabolic stress, tumor recruit surrounding endothelial cells and blood vessels for the development of new blood vessels, a process called as neoangiogenesis. Metabolic requirements are fulfilled by the formation of new blood vessels from the preexisting blood vessel. New blood vessels are dilated shows excessive branches and openings and improper basement membrane [54]. Narrow Band Imaging (Olympus Medical Systems Corporation, Tokyo, Japan) is a fiberoptic endoscopic visualization aid initially used for the gastrointestinal tract. It has also used for aerodigestive and urinary tract. Recently it is used for the diagnosis of OPMD and OSCC of the oral cavity. Working principle of the device is based on differential tissue penetration properties of light of different wavelengths. It enhances abnormal morphology and vasculature of mucosa and submucosa. Two optical filters in NBI mode select blue and green narrow bands of light with 450 and 540 nm wavelengths, respectively. Absorption peak corresponds to haemoglobin, thus highlighting the capillary bed and interpupillary capillary loop (IPCL). Blood vessels in the superficial layer appear brown while those in the deeper layer appear cyan enhanced by green light. Reflected light is captured by charge-coupled device (CCD) to produce NBI image [55]. Characteristic patterns of IPCL in leukoplakia, erythroplakia, chronic non-healing ulcers premalignant and malignant oral lesions can be effectively diagnosed with the help of NBI [56–58]. NBI is an effective aid for diagnosis of deeper potentially malignant lesions which is not possible with the help of normal white light. NBI is also effective (77%) for the diagnosis of second primary malignancies after surgery. Second primary malignancies can be detected in early stages which minimize the need of radical Surgery. Thus NBI will definitely helpful to improve functional outcomes if second primary tumors are diagnosed early [59]. NBI is certainly a prominent non-invasive visualization aid in the diagnosis of OSCC.

Raman spectroscopy

Professor Raman of Calcutta University discovered Raman effect for which was awarded with Nobel prize in 1930. When light interacts with matter most photons pass through it unchanged while some interacts with molecules in the matter. Only a tiny portion of photons among these (1 in 10^6-10^8) show phenomenon of inelastic scattering and discharge from material at a different wavelength called as Raman Effect. Lasers and charged coupled devices (CCDs) made possible the Raman spectroscopy (RS) of tissues possible by collecting weak Raman signals. RS is based on Raman effect which differentiates biological specimens on the basis of molecular details. RS is a high precision optical technique based on inelastic scattering of light by illuminating molecules. This non-invasive technique does not require use of reagents and dyes. It generates Raman spectrum based on molecular characteristics of the tissue and assigns characteristic peak to the corresponding molecule present in the tissue. It can be used in UV, visible and near-infrared wavelengths [60]. High spectral resolution at the molecular level enables detection of the exact location and borders of the lesion. Literature study showed *in vivo* and ex vivo use of RS. RS spectrum has been studied in exfoliated cells, cancer tissues and body fluids such as serum and plasma. Study conducted using portable Raman spectrometric instruments of the oral cavity using histopathology as a gold standard gives 94% specificity in discriminating normal tissue

from premaligant and malignant oral tissue [61]. Use of RS for ex vivo diagnosis of OPMD and OSCC by using a serum is minimally invasive and comfortable especially to screen high-risk population. Sahu *et al.* [62] found intense positive peaks of Raman spectra signifying high levels of DNA and proteins in the serum sample of buccal cancer and in situ tumor patient with respect to normal. *In vivo* RS analysis showed dominated protein bands in Raman spectra of OSCC. Premalignant lesions also showed a similar type of spectra. It is not possible to differentiate premalignant lesion from malignant on the basis of Raman spectra. Habitual tobacco users showed deviation from healthy control spectra [59]. RS spectra were obtained by using plasma, urine and saliva of healthy individuals, OSF, leukoplakia and OSCC patients. It discriminates OPMD and OSCC from normal control with significant accuracy of 78%, 90.5%, 93.1% and 97.4 % for blood, urine, saliva and tissue samples respectively [60, 61]. Dedicated instrument and stringent laboratory conditions limits the use of RS in large population screening.

Biomarkers of OSCC and OPMD

Cancer biomarkers are identifiable signature molecules produced by cancer cells or other cells in response to transformation and cancer development which could be useful for early diagnosis, prognosis, disease progression monitoring and treatment response. Various biomolecules such as DNA, mRNA, proteins, metabolites, mi-RNAs etc. act as a biomarker. The advancements in treatments of oral cancer such as surgery, chemotherapy and radiotherapy are not proven to be successful in the improvement of five-year survival rate of oral cancer patients, which is well below 50% [66]. This failure can be explained by late stage presentation of oral cancer. Classical Tumor, node and metastasis (TNM) classification is the basis for treatment planning and prognosis for OSSC in routine clinical practice which is insufficient to predict clinical outcomes in OSCC patients [67]. Precancerous lesions often go unidentifiable clinically only some are recognized as erythroplakia/leukoplakia etc. Malignant progression of such lesions requires years hence precancerous lesions are attractive targets for screening. Histopathological grading shows limitations in predicting risk of malignant potential and reliable prognostic markers are lacking for OSCC. Molecular analysis of such lesions has potential to predict risk of malignant transformation. Cancer research has revealed that there is a link between molecular and tissue-level changes that make malignant changes in the tissue causing disease progression. Biomarkers play a major role in distinguishing the presence or absence of disease. The "biomarker" is a biological molecule found in blood, body fluids, and tissues that is a sign of a condition of disease such as cancer. The biomarkers are classified on the basis of proteomics, genomics, metabolomic and Immunohistochemistry technique (fig. 1).

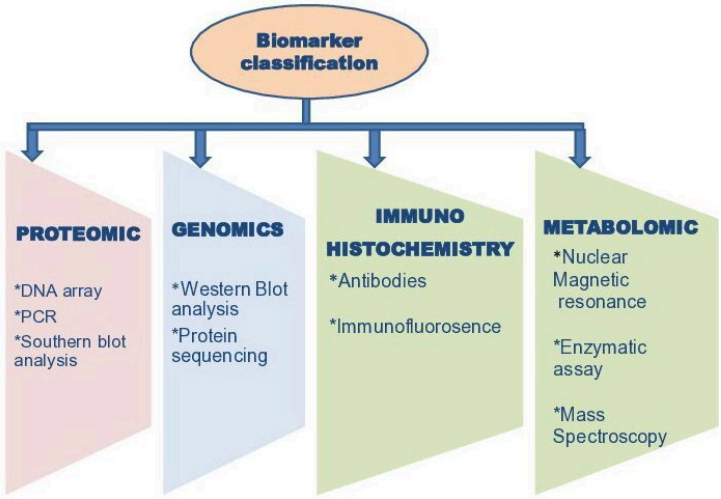

Fig. 1: Classification of oral cancer biomarkers

It include proteins, nucleic acids, peptides, enzymes, antibodies, metabolites, lipids, and carbohydrates. Biomarkers can be derived from one or a combination of the body fluids blood, serum, plasma, body secretions, stool, urine. Obtaining body fluids sample for biomarker investigation can be non-invasive, minimally invasive or least invasive. DNA/RNA extracted from blood, saliva will help to correlate and confirm the diagnosis, monitor the disease progression, or act as prognostic indicators in treatment. Early diagnosis and treatment of oral cancer can improve patient survival, which requires biomarkers that can be employed in routine clinical practice.

Proteomic techniques

Non-invasive detection methods are emerging as promising tools for the early detection of OSCC. Blood and saliva-based proteomic profiling is less invasive new practical approach. These body fluids are easily obtainable and contain characteristic biochemical signature molecules shed by oral lesions allowing detecting as a potential biomarker. Modernization of proteomic analysis techniques mass spectroscopy, liquid chromatography and protein-peptide labeling techniques identify salivary proteins with great precision. Proteins such as α-amylase, albumin, cystatins, hystatins, secretory-IgA, lactoferrin, mucins, lysozymes, proline-rich proteins, statherin and transferrin constitute 98% of total salivary proteins. Proteomic profiling of saliva from OPMD, OSCC and healthy individuals by means of SDS-PAGE coupled with LC-MS/MS identified around 1000 salivary proteins. Among them, 22 proteins are overexpressed in OSCC patients than healthy and OPMD group. Elevated Salivary Resistin (RETN) shows strong correlation with late stage primary tumors and could be a potential salivary biomarker for OSCC detection [68]. Enolase 1 have been significantly higher in saliva of OSCC suggesting its use as a biomarker [69]. Serum protein biomarkers are helpful for the assessment of the aggressiveness of tumors and lymph node metastasis. In research analysis levels of various serum proteins fibronectin, gelsolin, angiotensinogen (AGT) and Heptoglobin (HP) have been validated in node-positive tumors and negative tumors. These proteins are differentially expressed in node-positive and negative tumors [70].

The proteomic analysis of high throughput LC–MS/MS revealed the presence of 1256 proteins in saliva of a healthy individual. Out of total 3449 proteins, 139 salivary proteins have shown to be differentially expressed in oral cancer [71]. Liu *et al.* [72] validated 30 overexpressed proteins in OSF and OSCC tissues. Annexin A4 (ANXA4) and Filamin-A (FLMA) proteins are consistently upregulated while Fibrinogen alpha chain precursor (FGA) was downregulated from normal buccal mucosa tissue to OSF and OSCC, which shows prognostic significance. However, large multicentric

studies are required for the detailed investigation of this attractive method of OSCC biomarker investigation.

Genomic technique

Micro RNAs (mi-RNAs)

Mi RNAs are small non-coding RNAs (17–22 nucleotides in length) that regulate post-transcriptional gene expression during development, cell proliferation, metabolism and signal transduction by degradation of mRNAs. Mi RNAs have been studied extensively in serum, plasma, saliva, formalin-fixed and paraffin-embedded tissues (FFPE) and cell lines of OSCC as a potential biomarker. The presence of various types of circulating mi RNAs and its association with cancer manifestation, invasion and metastasis have been studied in various types of cancers [73]. Circulating miR-196a and miR-196b levels are significantly elevated in oral cancer and precancerous oral lesions. Combined detection of miR-196a and miR-196b can be a potential oral cancer panel plasma biomarker for early detection [74]. Plasma miR-223 levels increases in postoperative patients in which tumor relapsed in 6 mo. So, miR-223 is helpful for treatment monitoring and as a prognostic biomarker [75].

MiRNA profiling of OSCC patient's saliva high lightened its importance as a potential diagnostic biomarker for oral cancer. miR-125a and miR-200a levels are lowered in oral cancer patient saliva. miR-21,-31,-107,-138,-504,-10b, miR-16,-20a,-106b,-142-3p, miR-155,-423,-451, and let-7i levels are upregulated while miR-10a,-125b,-375 levels are down-regulated in formalin-fixed and paraffin-embedded tissues of squamous cell carcinomas [76]. Elevated miR-155 level is associated with tobacco chewing/betel quid habit in a RT-qPCR analysis of OSCC tissues of Indian patient study [77]. miR-1246 expression is increased in OSCC cell lines and tissues. Its high expression leads to poorer prognosis of OSCC patients, which suggests its importance as a promising prognostic biomarker [78]. miR-203 and miR-205 are uniformly expressed in lymph nodes of metastatic OSCC. Expression of these molecules can be evaluated in fine needle aspirate (FNA) biopsies for diagnostic purpose, sentinel lymph node examination during surgery, entire lymph node analysis after surgery along with histopathology as well as for patient follow up [79]. Many oncomiRs and their target m-RNAs have been studied as it acts as tumor suppressor miRs. OSCC Cell line study of the expression of MiR-494 revealed that it is emerging as a tumor suppressor miRNA as it repress the expression of HOXA 10 which is upregulated in OSCC [80]. MiR-506 target transcription factor GATA binding protein 6 (GATA6) and suppress the growth of tumor. It is downregulated in OSCC tissue and cell lines and is a attractive therapeutic target as its restoration is associated with tumor suppression. MiR-128 inhibits m-RNAs of H3f3b, BMI-1, PAIP2, BAG-2, and BAX. Overexpression of MiR-128 accelerates the apoptosis and inhibits cell proliferation of HNSCC (Head and Neck Squamous Cell Carcinoma), which suggests the clinical value of miR-128 as a therapeutic target [81]. Proto-oncogene Yes-1 (Tyrosine-protein kinase) expression is increased in cancer. Its upregulated expression is associated with tumor aggressiveness. MiR-203 suppress Yes-1 m-RNA and protein in oral cancer cells and induce apoptosis [82].

TNF-LTA Single nucleotide polymorphism

Various cytokines such as tumor necrosis factor (TNF)-α, TNF-β and lymphotoxin-αá (LTA) plays a role in the process of inflammation and tumor. These cytokines are encoded by TNFA and LTA genes located in the major histocompatibility complex (MHC) genes. A novel study in Indian patients with OSCC has identified two haplotypes of TNFA-LTA genes, which increases oral cancer risk. Patients with these haplotypes are genetically susceptible for oral cancer. It is a possible explanation for tobacco users who do not develop oral cancers. These genetic factors plays role along with environmental risk factors of OSCC and potential novel biomarker to calculate the predisposition of oral cancer in tobacco users [83].

Immunohistochemistry based biomarkers

Human papillomavirus (HPV) associated biomarkers

OSCC can be classified as HPV related and unrelated and shows different genetic and prognostic characteristics. Molecular signatures of HPV can be detected as HPV biomarkers which are helpful for management of OSCC patients. Immunohistochemistry (IHC) detection of $p16^{INK4A}$is well-established biomarker for HPV infection in OSCC. pRb inactivation by HPV E7 protein results in upregulation of CDKN2A gene which encodes cyclin-dependent kinase (CDK) inhibitor $p16^{INK4A}$[84]. Various molecular techniques with different specificity and sensitivity such as PCR, quantitative reverse transcription PCR, Southern blotting, dot blot hybridization, hybrid capture and in situ hybridization have been employed to detect HPV DNA in OSCC tissues and exfoliated cells [85]. Immunohistochemical detection of $p16^{INK4A}$ along with detection of HPV DNA is the gold standard for the detection of HPV in OSCC.

p53

p53 is tumor suppressor gene found to be deregulated in many types of cancers, including oral cancer. p53 is regarded as 'Guardian of the genome' which regulate cell cycle progression, cellular differentiation, apoptosis, DNA repair and maintain genomic stability. Immunohistochemical localization of p53 protein in normal tissues is difficult due to its high catabolic rate; however, its mutated version has a lower catabolic rate and tends to accumulate in the cells [86]. HNSCC with non-functional p53 are associated with increased resistance to conventional chemotherapies and radiation [87]. 50% oral cancer as well as leukoplakia/erythroplakia patients, shows p53 gene mutation and overexpression. p53 expression is positively correlated with the degree of dysplasia. Severely dysplastic lesions overexpress p53 than lesser dysplastic lesions. Dysplastic lesions with p53 overexpression shows high rate of malignant transformation [88].

Cyclin D1

Cyclin D1 gene (CCND1) encodes nuclear protein cyclin D1, a key cell cycle regulator that binds with cyclin-dependent kinases (CDK) 4 and 6. CDK 4/6 phosphorylate retinoblastoma protein (pRb) leads to its functional inactivation, which allows cell cycle progression from G1 to S. Cyclin D1 overexpression leads to loss of cell cycle control ultimately causes tumor genesis [89]. In a comprehensive meta analysis Zhao *et al.* [90] have found a significant positive correlation between overexpression of cyclin D1 with poor prognosis and detrimental clinicopathogical outcome. Its expression increases with increased tumor size, lymph node metastasis, tumor differentiation and clinical stage.

Epidermal growth factor receptor (EGFR)

EGFR is a transmembrane tyrosine kinase receptor that regulates cell differentiation and proliferation. It binds epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) and activate protein tyrosine kinase signaling. EGFR is expressed in low amounts in normal squamous cells [91]. EGFR overexpression in OSCC is caused due to amplification of proto-oncogene EGFR located at 7p12. It results in the proliferation and survival of cancer cells [92]. Overexpression of EGFR shows poorer prognostic outcomes in OSCC patients [93]. Sarkis *et al.* [94] have found EGFR expression in 87.5% cases and found a strong correlation with lymph node metastasis. Indian population-based study revealed positive EGFR signals in all OSCC and leukoplakia cases. EGFR expression has been lowered in leukoplakia tissue than OSCC [95].

Vascular endothelial growth factor (VEGF)

Angiogenesis is a hallmark of many cancers believed to stimulate tumor growth. VEGF is an angiogenic stimulator and also a prognostic predictor in OSCC. It is a dimeric glycoprotein having a molecular mass 34-42 kDa. Various normal and transformed cells secrete VEGF after growth factor stimulation and under hypoxic conditions. It is found in the tumor microenvironment, which results in tumor angiogenesis as well as induces endothelial cell proliferation, migration and survival [96]. 70 % OSCC tissues and 63.4% OPMD tissues showed positive immunohistochemical expression of VEGF. Higher expression has been observed in OSCC patients with lymph node metastasis, while controls tissues did not showed positive expressions [97].

Matrix metalloproteinase (MMPs)

Tumor cells detach from main tumor population, migrate and grow at secondary sites called as metastasis. Metastasis and tumor recurrence causes 90% mortalities in cancer patients. MMPs plays very important role in the process of metastasis by degrading components of extracellular matrix [98]. MMPs are extracellular zinc mettaloenzyme family of proteins comprises 24 members which took part in normal development and healing process. Three subgroups of MMPs Gelatinases (MMP-2 and-9), stromelysins (MMP-3,-10,-11), collagenases (MMP-1 and-13) are involved in the metastasis of OSCC. Increased expression of MMP1, MMP2, MMP3 and MMP9 have been observed in OSCC cancer tissue than normal. Therefore, Expression of MMPs have been studied as a potential biomarker for the assessment of tumor recurrence in primary cancers, including OSCC [99].

CD44

CD44 is a transmembrane glycoprotein that plays an important role in cell growth, adhesion, differentiation and motility. It is successfully used as a cancer stem cell marker to isolate cancer stem cells in various solid tumors. Inconsistent CD44 expression has been observed in OSCC. Savant *et al.* [100] have found that the expression of CD44 is elevated in OSCC with the increasing grades. However, it could be a useful prognostic marker in combination with oct4 and c-myc rather than CD44 alone. It is found to be overexpressed in cervical premalignant lesions and cervical carcinoma and effectively differentiates them with high sensitivity [101]. Soluble CD44 (solCD44) and total protein levels are significantly associated with the development of oral cancer in high-risk individuals. ELISA analysis of CD44 and total protein assay from oral rinses can be a predictive biomarker in the development of oral cancer [102].

Major complex class I-related chain A/B

Major Histocompatibility Complex Class–I chain-related antigen A and B (MICA/B) are the surface antigens expressed by all nucleated cells. MICA/B presents protein fragments of cytosol and nuclear origin at the cell surface called antigen representation. MICA/B are highly polymorphic proteins induced in stressed, damaged and transformed cells and gives 'kill me' signal to NK, CD8 and γδ T cells by natural killer group 2D (NKG2D) receptor engagement. These molecules act as ligand for many immune cells and proposed to play an important role in tumor immunosurveillance. MICA/B is upregulated in response to various cellular stress stimuli such as CMV infection, inflammation or malignant transformation. They are shown to be expressed by epithelial tumor cells of the lung, colon and renal. MICA/B are the ligands of NKG2D receptors present on the natural killer andT cells (fig. 2).

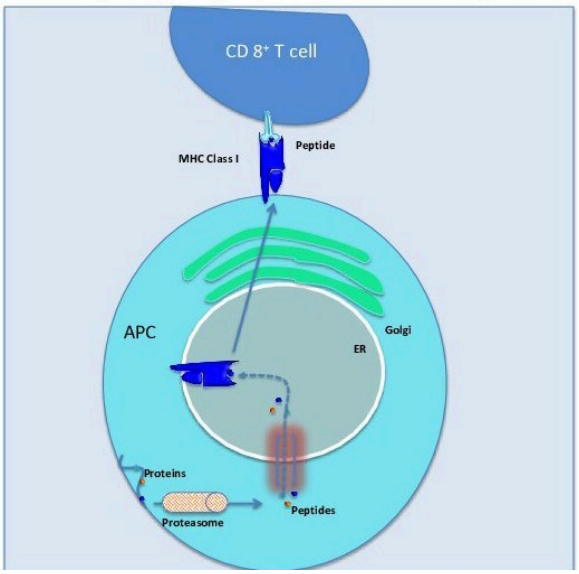

Fig. 2: Hajor histocompatibillty complex classs-1 chain related antigen A and B

Binding of NKG2D with MICA activates the NK cells, co-stimulates γδT cells and $CD8^+$T cells. The MICA-NKG2D system participates in epithelial tumor immune surveillance. Many tumours have evolved stratergies to evade the immune system by MIC shedding from cell surface. Endopeptidases called as metalloprotenases are responsible for the cleavage of MICA α1α2α3 domain in many tumor cell lines [99]. In a large sample size study of about 512 individuals carried out by Stefan Holdenrieder *et al.* revealed significantly higher levels of sMICA in sera of malignant cancer patients (161 pg/ml) while intermediate sMICA levels (84 pg/ml) have been observed in patients with benign diseases. Healthy individuals exhibited lower levels of sMICA (30 pg/ml). sMICB levels did not differ significantly from normal control but significantly increased in stage IV OSCC [100].

CONCLUSION

OPMD such as luekoplakia and OE have high malignant transformation potential. Indigenous tobacco habits can be linked to the development of OPMD. There is an urgent need to establish mechanism to screen the high-risk population for OPMD. It is of immense importance that diagnostic availability of novel biomarkers with high diagnostic and prognostic value and low-cost monitoring. Prevalence data of OPMD is also not significant enough as most of the studies are hospital-based. Real prevalence may differ in the general population in India. Large population based randomized case-control studies are required to obtain prevalence data. This data will definitely help to design new strategies of oral cancer prevention of National Cancer Control Program.

OVERVIEW

In India, oral cancer incidences continued to increase in young men and women. Currently, there are consencious for using marker that can be used for early detection and diagnosis of OSCC. Future research efforts in India aimed at Multicentric studies to identify the biomarker for early detection and diagnosis, and ultimately the survival of OSCC patients. It will generate the detail evidence of population based screening program in India and investigate the different ways of public awareness campaigns, practitioner education, and policy development to enhance the prevention of OSCC. The proposed theory of current knowledge will benefits to investigate the new ways to examine for high-risk populations for OSCC and educate them for oral health care. It will be the most

effective and appropriate ways of raising public awareness of OSCC and its risk factors.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors have no conflicts to declare.

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