Review Article

Oral cancer diagnostics: An overview

ABSTRACT

Cancer was first mentioned in medicine texts by Egyptians. Ancient Indians studied oral cancer in great detail under Susruta. Cancer has continued to be a challenge to physicians from ancient times to the present. Over the years, cancer underwent a shift in management from radical surgeries toward a more preventive approach. Early diagnosis is vital in reducing cancer-associated mortality especially with oral cancer. Even though the mainstay of oral cancer diagnosis still continues to be a trained clinician and histopathologic examination of malignant tissues. Translating innovation in technological advancements in diagnostic aids for oral cancer will require both improved decision-making and a commitment toward optimizing cost, skills, turnover time between capturing data and obtaining a useful result. The present review describes the conventional to most advanced diagnostic modalities used as oral cancer diagnostics. It also includes the new technologies available and the future trends in oral cancer diagnostics.

Keywords: Autofluorescence, brush biopsy, oral cancer, tissue reflectance, transepithelial cytology

INTRODUCTION

The global incidence of cancer stands at 18.1 million new cases, 48.4% of which occur in Asia. This disease claimed the lives of approximately 9.6 million people, in 2018. In 134 out of 183 countries of the world, Cancer is the leading cause or the second-most common cause of early death, killing one in six people. As life expectancy increases and populations evolve and changes in epidemiology occur, cancer will only continue to be a scourge.[1]

India accounts for a third of the total cases of oral cancer in the world. The online database Globocan that provides the incidence and mortality for 36 major cancers worldwide pegs new cases of oral cancers in India at 119,992 leading to death of 72,616 Indians, annually. This means of all the cancers affecting India, oral cancer alone accounts for 30% cases. According to the Indian Council of Medical Research, more men than women die of oral cancer.[2]

In India, Population Based Cancer Registries (PBCR) are maintained to follow the epidemiologic trends in the disease across the country. In the last report prepared by pooling the data from each PBCR, the Indian Council of Medical Research reported that Bengaluru and Chennai had the highest age adjusted rate of cancers of all sites with more women affected than men. Among men, cancers involving the tongue showed the highest age adjusted rate in East Khasi Hills District from Meghalaya (11.7%) whereas among women this was noted to be highest in Bhopal (18.1%). Cancers of mouth showed highest age adjusted incidence at 18.1% among men from Ahmedabad Urban Population Registry followed by those from Bhopal at 14.3%. Among Indian women, the highest incidence of cancers involving the mouth were noted in East Khasi Hills District (9.1).[3]

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Received: 27 June 2020, **Revised:** 02 September 2020, **Accepted:** 10 December 2020, **Published:** 13 December 2021

Access this article online

Website: www.njms.in

DOI: 10.4103/njms.NJMS_130_20

Quick Response Code

How to cite this article: Chaurasia A, Alam SI, Singh N. Oral cancer diagnostics: An overview. Natl J Maxillofac Surg 2021;12:324-32.

The overall 1-year survival rate for patients with all stages of oral cavity and pharynx cancers is 81%. The 5- and 10-year survival rates are 56% and 41%, respectively.[4] The term "head and neck cancer" refers to neoplasm arising from below the skull base to the region of thoracic inlet. It includes mucosal surface of the lip, oral cavity, pharynx, larynx, nose, Para-nasal sinuses, salivary glands, and cervical gland deposits. There are many histological types but squamous cell carcinoma is the most common one.[5] Oral cavity extends from lips to the junction of hard and soft palate superiorly and the circumvallate papillae of tongue inferiorly and includes commissures, tongue, floor of mouth, gingivae, buccal mucosa, retromolar trigone, and hard palate.[6] Oral cancer is a nonspecific broad term encompassing all neoplasms that involve the structures in this anatomical region. The most common cancer involving this area is the squamous cell carcinoma followed by verrucous carcinoma.

The leading preventable cause for cancers in oral cavity is tobacco use. Fact Sheet of the India chapter of Global Adult Tobacco Survey 2 (2016–2017) shows that prevalence of tobacco use stands at 28.6%, i.e., 42.4% of males and 14.2% of females which means that 266.8 million adults in India use tobacco in one form or another.[7] Other than chewing or smoking tobacco important predisposing factors for oral cancer are age, male gender, alcohol, sun exposure, ionizing radiation, betel (areca) chewing, immunosuppression and graft versus host disease, infections with human papilloma virus (HPV), low socioeconomic status, diet, certain occupations, and poor oral hygiene.[8] This review enlightens all the diagnostic modalities/technologies used in diagnosis of oral cancer very ranging from conventional to most advanced one.

HISTORY AND BACKGROUND OF DIAGNOSIS OF CANCER

The first recorded mention of oral cancer is found in ancient Egyptian medicine wherein the Ebers Papyrus dated between 1600 and 1550 BC ancient Egyptian practitioners of medicine mention several lesions likely to be cancer especially in the last part titled Treatise on Tumors where the term "bnwt" is used to describe an "eating ulcer on the gum." The ancient Indian text "Sushruta Samhita" dating to mid-first millennium BC in very likely the first effort to classify body tumors. Interestingly, chewing of betel quid one of the most established risk factors of oral cancer was already documented in this book. Sushruta Samhita mentions tumors and metastases separately as arbuda and arbudam, respectively. Furthermore, mentioned are the terms "Mánsaja" for lip cancer, "Mahá-Saushira" for alveolar cancer, "Aryuda" for palatal cancer, "Alása" for cancer of the tongue's base and "Adhjihva" for cancer of tongue's tip, Rohini, Sataghni and Valása to describe pharyngeal and hypopharyngeal tumors, "Kaphaja Rohini, Valaya and Giláyu" types for tumors of the postcricoid and esophagus, "Svaraghna" for laryngeal tumors. The abundance of detailed descriptions and mentions of oral pathology and the attention of the authors focused on these kinds of cancer lead us to think how these oropharyngeal diseases and tumors must have been quite common and diffused among Indian people of that time reflecting a similar condition to the current situation.[9]

The surgeon physician, Democedes of Croton was probably the first physician to treat cancer. He is credited to have cured queen Atossa of a chronic growth or swelling of breast which Ewing in 1940 mentions as likely to be a cancer and contested by some authors to be inflammatory mastitis. The point to be noted is that Democedes though seen to favor conservative management relied on excision in the case of this disease.[10] By 1969, however, as Richard Nixon were called on to cure cancer and as media demanded a "moon shot," the rather ambitiously named Citizens Committee for the Conquest of Cancer was confident enough to hope for an imminent cure. And yet nearly half a century later as Madeline Drexler memorably wrote in Harvard Public Health Magazine last year the fundamental flaw in that approach was underestimating prevention of cancer.[11] The shift in paradigm of the management of cancer is obvious from dramatic cures toward less dramatic but more impactful prevention.

The backbone of prevention is early diagnosis. In 2005, the WHO Health assembly passed a resolution on cancer prevention and control stressing on the importance of prioritizing preventable tumors and exposure to risk factors such as tobacco, unhealthy diet, and alcohol abuse with special emphasis on cancers amenable to early detection and treatment specifically oral cancer, cancers of cervix, breast, and prostate. The WHO Global Health Programme is committed toward oral cancer prevention and among other objectives also toward integrated approaches in prevention and health promotion. In 2007, for the first time in 25 years the World Health Assembly passed a resolution for Oral health. In clause WHA60 A16 member states are requested to ensure prevention of cancer as an integral part of national cancer-control programs and to involve oral health professionals or primary health-care personnel with relevant training in oral health in detection early diagnosis and treatment.[12]

THE DIAGNOSIS OF CANCER

Predisposing causes of head and neck cancers are obscure but in many cases related to tobacco smoking and chewing, age, male

gender, alcohol, sun exposure, ionizing radiation, betel (areca) chewing, immunosuppression, and graft versus host disease, infections with HPV, low socioeconomic status, diet, certain occupations, and poor oral hygiene appear to increase the risk for oral cancer. Among the premalignant condition are leukoplakia, erythroplakia, oral submucous fibrosis, melanoplakia, lichen-planus, sclerosing hemangioma. Five percent–37% of leukoplakias develop into cancer as reported in various studies.[13,14] The risk of malignant transformation has been reported to be between 6.6% and 36.4% although a recent meta-analysis indicated a rate of 12.1%.[15]

Commonly, the tumor spreads by direct route along the facial planes to involve adjacent soft tissue structure and lymphatics. Hematogenous spread occurs late in the course of disease and lead to distant metastasis. Lung is the most common site of involvement in 50% of metastasis cases. Lymphatic involvement depends on various factors such as histology, grade, size, and site of primary tumor. Common presenting features of head and neck cancer are dysphagia, odynophagia, otalgia, hoarseness of voice, mucosal irregularity, ulceration, pain, weight loss, and presence of unexplained neck mass. Predominant symptoms vary with the site of involvement. Swellings/thickenings, lumps or bumps, rough spots/crusts/or eroded areas on the lips, gums, or other areas inside the mouth. The development of velvety white, red or speckled (white and red) patches in the mouth unexplained bleeding in the mouth, unexplained numbness, loss of feeling or pain/tenderness in any area of the face, mouth or neck, persistent sores on the face, neck or mouth that bleed easily and do not heal within 2 weeks. A soreness or feeling that something is caught in the back of the throat, difficulty chewing or swallowing, speaking or moving the jaw or tongue, hoarseness, chronic sore throat or change in voice or ear pain a change in the way your teeth or dentures fit together or dramatic weight loss.[16]

DIAGNOSTIC AIDS FOR ORAL CANCER

For the purpose of this article, we have classified the available diagnostic aids based on the main underlying principle of their functioning [Table 1].

Table 1: Diagnostic aids and their functioning principle

Principle	Diagnostic aid
TF	Use of direct light Visual examination with histopathological examination STOP tool for screening Vital staining Toluidene Blue Lugol's Iodine Chemiluminescence Vizilite LED light source Microlux/DL™ Orascoptic EVINCE LIAF
AF	VELscope
Combined use of TF and AF	Identafi 3000
Transepithelial cytology	Brush Cytology Oral CDx
PCR Biomarkers Proteomics Microbiology	Salivary studies
Confocal scanning light microscopy	IVCM
Optical coherence tomography	
Fluorescence lifetime imaging	
Artificial intelligence	

PCR: Polymerase chain reaction, TF: Tissue reflectance, AF: Autofluorescence, LIAF: Laser-induced autofluorescence, IVCM: *In vivo* confocal microscopy

VISUAL EXAMINATION WITH HISTOPATHOLOGICAL EXAMINATION AND THE STOP TOOL

The gold standard for diagnosis of oral cancer is tissue biopsy with histological assessment. This technique requires a trained health professional and is invasive, painful, expensive, and time consuming.[17] In 2012, Thomas *et al.* proposed a simple tool for opportunistic general health care screening forwarding the suggestion that all potentially malignant disorders be considered under the umbrella term of MD-OEDr, i.e., mucosal disorders with oral epithelial dysplasia risk. The STOP has four items-white lesion, white lesion with ulcer, mucosal change and persistent ulcer with scores ranging from 0 to 3 and color coding of risk from green being low risk, blue as general risk to be guarded with caution while yellow, orange, and red signify significant high and severe risk for progression to cancer. This tool was tested on a sample of 255 subjects and sensitivity was found to be 96.6% and specificity to be 99%. The positive predictive value (PPV) of the tool is 96.6 and negative predictive value (NPV) is 99.9 with a reliability coefficient of 0.874.[18] Brocklehurst *et al*. (2013) published the result of sifting through 3239 records in a systematic review of programs for the early detection and prevention of oral cancer. One important finding was that visual examination reduced mortality from oral cancer when used within a targeted screening program.[19] As such we note that in areas with paucity of trained health professionals the STOP tool can be used for screening and since it has been shown to have a high diagnostic odds ratio, i.e., it differentiates well between the diseased and nondiseased. It can aid in decision making by sending those at high risk for oral examination by experts.

VITAL STAINING

Vital staining is staining live tissues/cells and it works on the principle of metachromasia. In 1953 Slaughter *et al*. forwarded the concept of field concretization-a zone of epithelial dysplasia surrounding early oral squamous cell carcinoma (OSCC).[20] It was not long before investigators put two and two together and started working on finding ways to stain potentially malignant tissue which may look innocuous clinically.

LUGOL'S IODINE

Iodine staining of mucosa to identify cancerous lesions was first reported by Schiller in 1933 who used it for diagnosing cervical cancer.[21] Lugol's Iodine infiltrates and reacts with glycogen, to produce a black brown stain, and can confirm both histological and molecular margins in OSCC. The more keratinized the mucosa the more the staining with iodine. Xiao *et al*. hypothesised that increase in glycolysis noted in high cell proliferation depletes glycogen within the epithelium, which leads to failure of staining in malignant mucosa.[22] The sensitivity of this investigation is reported to be 87.5% and specificity to be 84.2% by Epstein *et al*. They reported a PPV of 0.921 and a NPV of 0.762.[23]

TOLUIDENE BLUE

First discovered by WH Perkin toluidine blue was used primarily as an industrial dye. Richart in 1963 used it to stain uterine cervix cancer for the first time in live human patients.[24] Five years later, Strong, Vaughan, and Incze, suggested it be used for identifying malignant oral lesions.[25] Toluidene blue binds to nucleic acid in tissues but binding decreases with development of cell and with fall in pH.[26] Thus, it is acidophilic and preferentially stains acidic tissue components (nucleic acids). In practice, a 1% solution of toluidine blue at a pH of about 4.5 is used for oral rinsing or swabbing followed by a rinse or swab of 1% acetic acid. Oral mucosal sites which retain the blue color are evaluated for malignant change. Epstein, Scully and Spinelly calculated the sensitivity of toluidine blue stain to be 92.5% and specificity to be 63.2%, with a PPV of 0.841 and a NPV to be 0.800.[23]

Interestingly, the same authors also evaluated the usefulness of both dyes when applied together. When toluidine blue was used with Lugol's iodine sensitivity was reported to be 85.0% and specificity to be 89.5%. PPV was noted to be 0.944 and NPV to be 0.739. Taking note of the fact that toluidine blue proved more sensitive than specific and Lugol's iodine was shown to be more specific than sensitive. The authors advised using these dyes as diagnostic aids for patients at risk or for patients with suspicious lesions rather than as screening measures in general population.

VIZILITE

Also called lumenoscopy, Vizilite produced by Zila Pharmaceuticals Phoenix Arizona was approved for clinical use by US FDA in 2002. Based on chemiluminescence, this is a single use kit which involves dehydrating the oral mucosa with acetic acid and exposing it to chemiluminescent light from a dye in a capsule. The capsule contains aspirin in outer shell and hydrogen peroxide in inner shell is flexed until the latter breaks through and reacts with a luminescent dye to produce light in the range of 430–580 nm which lasts for about 10 min and its reflectance is used to evaluate the surface of oral mucosa. A later modification Vizilite Plus uses toluidine blue to enhance visual effect. Normal epithelium appears blue and dysplastic epithelium appears white (acetowhite).[27] In a study done by Mehrotra *et al*. in a cross-sectional survey of 102 patients Vizilite with Toluidene blue performed rather dismally picking no lesions at all. Its sensitivity was thus at 0% and specificity at 75.5%.[28] Kämmerer *et al*. working on a smaller sample size of 44 patients with 50 oral lesions in their evaluation found Vizilite when used without toluidine blue to have a sensitivity of 100%, specificity 30%, a PPV of 26% and NPV of 100%. Combined with toluidine blue, the sensitivity fell to 80% but specificity rose to 97.5%, with a PPV of 89% and an NPV of 95%.[29]

MICROLUX/DL™

Microlux/DL™ uses a battery powered light-emitting diode (LED) as a diffuse light source. A rinse with acetic acid is advised to break the glycoprotein film on oral mucosa to aid better visualization of lesions as in case of Vizilite. Dysplastic tissue takes on a whitish color increasing its visibility in comparison with normal healthy mucosa. McIntosh *et al*. examined 50 patients with this device and on comparison with histopathological examination found its sensitivity to be 77.8%, specificity 70.7% with a PPV of 36.8% and an NPV of 93.5%.[30]

ORASCOPTIC

Manufactured by Orascoptic Middleton (USA), Orascoptic is similar to Microlux/DL. There is a paucity of diagnostic parameter studies available on searching in commonly available search engines.

EVINCE

This is a novel device from MM Optics, São Carlos (Brazil). It works on the principle of autofluorescence and uses an LED

to produce light at a wavelength of 400 nm for examination of oral cavity with the help of an optical filter. In a pilot study (Simonato 2016), Evince showed a sensitivity of 100% and a specificity of 46% with a positive predictive (value [PPV] 22.22% and NPV of 53.33% in identifying epithelial dysplasia. The sample size being 15 subjects with 11 males and 4 females limits the usefulness of this study.[31]

LIAF IMAGER

Laser Induced Autofluorescence (LIAF) uses an LED to generate emission spectra in the range of 420–720 nm for scanning. At 500 nm autofluorescence is noted in healthy oral mucosa whereas malignant mucosa shows autofluorescence at 635, 685 or 705 nm. Mallia *et al*. proposed a reference standard for using LIAF for early detection of oral cancer. Using a diode laser with spectrum at 404 nm, they based their results on 14 anatomical sites of 35 healthy controls and 91 sites of 44 patients with dysplastic or anaplastic lesions. They suggest F500/F685 ratio showed 100% sensitivity and specificity for discriminating between normal and premalignant or malignant tissue.[32] Yan *et al*.(2017) used *ex vivo* samples of oral cancer to evaluate a portable LIAF imager at 365 nm wavelength measuring 221 points in 31 patients and reported a sensitivity above 84% and specificity about 76% with an accuracy of approximately 80% supporting the use of LIAF for noninvasive examination of oral cancer.[33]

VELSCOPE

Velscope or Visually Enhanced Lesion scope manufactured by LED dental, white rock, British Columbia (Canada) is an autofluorescence based hand held device which helps in screening suspected lesions based on differences in fluorescence pattern of healthy and dysplastic mucosa. Normal squamous epithelium of oral mucosa autofluoresces at 460 nm whereas malignant epithelium shows peak intensity at 635 nm. This difference in intensity of autofluorescence is seen with the help of Velscope.[34] While the premise is theoretically sound clinically the experience of investigators is varied.[35] Hanken *et al*. reported a sensitivity of 22% and a specificity of a mere 8.4% in their single-blinded evaluation of a group of 120 patients where they compared how Velscope compared against conventional white light. Sawan and Mashlah, evaluated 748 patients with Velscope and performed histopathological examination of lesions identified as being high suspicion. They calculated the sensitivity to 74.1% and specificity to 96.3%.[36] In the study reported earlier done by Hanken *et al*. comparing vizilite and velscope, it was found that velscope identified 6 out of 11 dysplasia and one malignancy pitching its sensitivity at 50% and specificity at 38.9%.[35] The authors further suggested caution in interpretation of results from these devices owing to the high rate of false negatives.

IDENTAFI 3000

Increased vascularization, a hallmark of malignancy leads to changes in reflectance between normal and potentially malignant tissue and this spectroscopic difference is the basis for the Identafi device which is manufactured by Star Dental-Dental EZ, Lancaster (USA). The device is portable and multiuse.[37] Based on the underlying principles of autofluorescence and confocal microscopy, Identafi 3000 uses fiber-optic light sources housed in a mouth mirror like casing and three wavelengths are used to examine the mouth, white conventional light-405 nm for autofluorescence and 445 nm green amber light for spectroscopic differences. Zuluaga *et al*. did a study involving 120 subjects across 4 centers and reported that in one of the four cohorts Identafi showed "perfect predictive value" with a PPV at 60% when loss of autofluorescence was used to differentiate between healthy and dysplastic or anaplastic tissue. They mentioned that this data was in accordance with other cohorts they studied but definitive percentages are wanting.[38] Lane *et al*. evaluated the device in 2012 but did not provide a sensitivity or specificity although they do stress on NPV and PPV which they also did not provide in their 2012 article.[39] Based on its underlying principle, Identafi looks promising but research is required to evaluate what it brings to the table regarding cancer screening.

BRUSH CYTOLOGY

In the 1940s, the study of exfoliated cells of mucosa to diagnose dysplastic or malignant changes gained ground due to the efforts of Papanicoloau and Traut who worked on collection and staining of these for gynecologic diagnosis.[40] In 1951, Montgomery and von Hamm, used this technique for lesions in oral cavity.[41] A special brush is used to collect a complete transepithelial sample and the cells thus obtained are stained with a modified Papanicolaou test and studied microscopically using a computer based imaging system.

Scheifele *et al*. evaluating their experience of 103 oral brush biopsies in 80 patients reported a sensitivity of 92.3% and a specificity of 94.3% with a positive likelihood ratio of 16.2 and a negative likelihood ratio of 0.08.[42] Mehrotra *et al*. used oral brush biopsy without computer assisted analysis and reported a sensitivity of 76.8% and a specificity of 93.3%.[43] Mehrotra *et al*. in their review of data available

regarding brush biopsy advised of caution because even though the sensitivity and specificity of brush biopsies on their own are promising the size and topography of oral cavity do not permit a complete examination of the entire mucosa.[44]

DNA PLOIDY AND QUANTIFICATION OF NUCLEAR DNA CONTENT

Stephenson *et al*. evaluated the relation between DNA ploidy and stage of prostatic cancer in 366 patients of the disease by studying DNA specimens obtained from archived paraffin-embedded tumor samples in metastatic nodes. They found out that flow cytometric DNA measurements were a strong predictor of survival for D1 stage of prostate cancer.[45] Pekta *et al*. took 44 samples from oral cavity of 22 patients and reported that 20 subjects were diploid (90.9%) while 2 showed aneuploidy (9.1%) whereas when only malignant lesions were taken into account diploid samples were 83.3% and aneuploid ones were 16.7%. They also noted a statistically significant difference in nuclear perimeter, DNA content and DNA index values among other findings.[46] The samples can be obtained by brush biopsies but the information on the role of DNA ploidy is limited regarding screening for oral cancer in the clinic and further studies are required to ascertain its future potential in cancer diagnostics.

POLYMERASE CHAIN REACTION BASED DIAGNOSTIC AIDS

Polymerase chain reaction (PCR) with its ability to amplify even tiny amounts of genetic material has swum back into the picture during the ongoing COVID-19 pandemic. In oral cancer however it offers an efficient method that requires noninvasive simple sampling and can yield information on the genetic status of lesions. It helps in finding mutated oncogenes and can potentially serve as an important detection tool for diagnosing oral cancer.[47] The technique is highly sensitive in itself but has the major drawback of minor contaminations causing difficulties in interpretation of results. One area it shows promise is in isolating the DNA of HPV virus with diagnostic kits available commercially.[48]

Oropharyngeal cancer due to HPV infection shows degradation of p53, inactivation of retinoblastoma RB pathway and upregulation of P16 whereas that due to tobacco exposure has mutation of TP53 and downregulation of CDKN2A that encodes for P16. Detection of such changes in DNA by PCR offer a path to diagnose cancers of the oropharynx with an eye on the course of disease and patient morbidity.[49]

SALIVA-BASED ORAL CANCER DIAGNOSTICS BIOMARKERS, PROTEOMICS, MICROBIOTA

Liao *et al*. published an interesting study in which they forwarded the idea that mutation of p53 codon 63 was present in saliva and can be used as a molecular marker for OSCC.[50] Jiang *et al*. studied oral rinse samples from 94 patients of confirmed squamous cell carcinoma of head and neck region and the oral rinse samples of 656 patients they were screening for the same disease. They reported that significant correlation between rise in mitochondrial DNA content in saliva and head and neck cancer, respectively. The head and neck squamous cell carcinoma was an independent predictor of elevated mitochondrial DNA in saliva.[51] Researchers at university of California Los Angeles discovered 309 distinct proteins in human saliva. They found that interleukin (IL-8) and thioredoxin could differentiate between patients with and without oral cancer. While thioredoxin is still under evaluation, they noted that IL-8 at a cut off titer of 600 pg/ml was significantly raised in saliva of patients with oral cancer showing a receiver operating characteristic (ROC) of 0.95 a sensitivity of 86% and a specificity of 97%. Furthermore, interestingly, patients of oral cancer also showed a significantly raised IL-8 mRNA content than normal subjects. The study group further noted an additional 3000 human mRNAs in cell free saliva of normal subjects. Out of these four mRNAs in combination-ornithine decarboxylase antizyme-1, spermidine acetyltransferase, IL-8 and IL-1β β can identify oral cancer with an ROC of 0.95, sensitivity and specificity of 91%. In their experience of 8 more independent clinical studies, they report a consistent accuracy rate of 85% for seven salivary mRNA biomarkers.[52] In a nonrandomized study (Mager 2005) involving 229 OSCC free and 45 OSCC patients salivary counts of 40 common oral bacteria were noted and 3 were found to be significantly raised in the diseased and the disease-free population namely *Capnocytophaga gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis*. High salivary counts of these three show a diagnostic sensitivity of 80% and specificity of 82% with a PPV of 80% and NPV of 83%.[53]

Schlussel *et al*. compared endothelin receptor type B (EDNRB) hypermethylation in salivary samples of 191 patients with methylation specific PCR. They compared it with expert clinical examination for screening precancers and cancers of oral mucosa. They showed a significant association between premalignancy and malignancy of oral mucosa with the genes HOXA9, EDNRB and DCC but noted that histopathological agreement was only in case of EDNRB. In their experience clinical risk assessment by experts identified dysplasia/ cancer with 56% sensitivity and 66% specificity with a 95%

confidence interval whereas on comparison EDNRB and DCC taken together showed a lower sensitivity of 46% and a higher specificity of 72%. However, when both expert opinion was aided by salivary rinse study of EDNRB and deleted in colorectal cancer sensitivity rose to 73% and 69% respectively and specificity became 51% and 59% respectively.[54]

IN VIVO CONFOCAL MICROSCOPY

In vivo confocal microscopy is an emerging noninvasive imaging and diagnostic tool which enables analysis of surface microstructure. Marvin Minsky was the first person to suggest the usefulness of this technology and holds the patent for it. It uses a slit scanning microscope to scan multiple points in parallel in section (one image), volume (multiple images at a selected depth), and a sequence scan (1–30 frames of varying depths presented as moving images). It is unique in that it can visualize imaging of moderately opaque tissues and help see dynamic processes of say inflammation and healing.[55] Because of this it was first used in ophthalmology and from there adapted for use in oral cavity. Gerger *et al*. in a sample of 117 melanocytic lesions and 45 non melanocytic ones reported a PPV of 94.22% when differentiating between melanoma and all other lesions and 96.34% when diagnosing malignant skin lesions. They also reported a 100% PPV for basal cell cancers and seborrheic keratosis.[56] Pierce *et al*. used multimodal optical imaging system and reported results of evaluating 100 sites in 30 subjects. In their experience, it got 98% of anatomically normal sites correct and correctly identified 95% of dysplastic and anaplastic lesions.[57]

OPTICAL COHERENCE TOMOGRAPHY

In optical coherence tomography (OCT), light is sent into tissues and it is reflected back just as sound waves are used in ultrasonography but unlike sound waves light travels fast and therefore direct measurements as with ultrasound are not feasible in practice. Hence, the light beam is split with half the beam directed into tissue being examined and the other half toward a reference mirror. Light is reflected from mirror and tissue specimens and reflected beams undergo interference. This phase difference is picked up by radial scanner and a two-dimensional image is generated. Alternative light sources are being explored to improve the resolution of these images. Advancement in this technology called the "Femtosecond transillumination tomography" has been shown to image up to a depth of 15 mm in experiments.[58] Tsai *et al*. scanned oral cavity of 32 patients and reported that for relative alpha scan sensitivity of detecting epithelial hyperplasia was 18.8%, for moderate dysplasia 50% and for oral squamous cell carcinoma 46.7% and a specificity of 81.3%. When they used relative T scans, the sensitivity of epithelial hyperplasia and moderate dysplasia were respectively 75% and 83.3% specificity for epithelial hyperplasia in T scan group was 25%.[59] Wilder-Smith *et al*. compared OCT with histopathology in 50 patients and reported sensitivity in detecting oral mucosal dysplasia and malignancies to be 93.1%. Specificity was 93.1% for detecting dysplasia and carcinoma *in situ* but 97.3% for detecting OSCC.[60]

FLUORESCENCE LIFETIME IMAGING

In 1992, a new fluorescence imaging methodology was described which is analogous to magnetic resonance imaging (MRI). While in MRI the lag in proton relaxation times at each point creates a contrast for obtaining an image in fluorescence lifetime imaging (FLIM) local environment of the tissue affects the lifetime of a fluorophore. This difference in fluorescence lifetimes is picked up by a gain-modulated image intensifier of a slow scan CCD camera. These biochemical tissue map information is processed by a computer and an image is obtained, thus allowing both chemical and physical imaging of samples. In FLIM, image contrast is built on the lifetime of a fluorophore so it is not affected by local concentration or even the intensity of the fluorophore.[61] Sun *et al*. studied 26 oral sites in 10 patients and reported head and neck squamous cell carcinoma showed shorter average lifetime and less than half the fluorescence intensity than that of healthy tissue demonstrating a role for FLIM in intraoperative surgical procedures.[62]

In a study of hamster cheek pouch to evaluate accuracy of OCT and FLIM, complimentary information obtained from both diagnostic aids when used together resulted in highest sensitivity and specificity. Combined OCT and FLIM use showed a sensitivity of 88.2% and specificity of 92% for benign lesions while for precancers a sensitivity of 81.5% and specificity of 96.0%. Combined use yielded a sensitivity of 90.1% and a specificity of 92.0% for cancerous lesions in this animal model study.[63]

ARTIFICIAL INTELLIGENCE

With its ability to process large volumes of data for decision making in a quantifiable, reproducible, and customized way, artificial intelligence is a powerful technology to reckon with. AI-based programs can detect subtle variations lost on human observers and have the capability to combine data from multiple sources such as images, genomics, proteomics, electronic health records, and even social networks into a cohesive whole. This streamlines predictive models and helps to integrate diagnosis.[64] Machine learning can improve

decision making in cancer diagnosis but there is not enough validation to include it in clinical practise at present.[65] Jeyaraj and Samuel Nadar reported using partitioned deep convolution neural network on hyperspectral images of patients to develop algorithm for computer aided diagnosis of oral cancer. They calculated a sensitivity of 94% and a specificity of 91% in a data set of 100 images with 91.4% accuracy in differentiating between cancerous lesions and benign ones. In another data set, they noted 94.5% accuracy in discriminating between normal tissue and malignant tissue.[66]

CONCLUSION

The flurry of technological advances should not detract us from our end goal of providing affordable, equitable and noninvasive means of cancer diagnosis which compromises neither on quality nor on ease of access. Translating innovation in technological advancements in diagnostic aids for oral cancer will require both improved decision making and a commitment toward optimizing cost, skills, turnover time between capturing data and obtaining a useful result. In addition, any machines or gadgets to be used should be portable, durable, and easy to sterilize between patients.

Theoretical and laboratory research in technology jumps by leaps and bounds every day. Clinical research will need to match that pace. Studies on oral cancer diagnostics need to be standardized to include the test measures of sensitivity, specificity, false positives, false negatives, PPVs, and NPVs. This will help clinicians make informed decisions about new devices available in the market.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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