

REVIEW ARTICLE

Conventional and Advanced Diagnostic Tools in Oral Cancer with Emphasis on Role of Dentist and Early Detection

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ABSTRACT

The prevalence of oral cancer is increasing worldwide day by day. Those who suffer from oral cancer have low survival rate compared with other cancer, mainly due to delay in the diagnosis, metastases, and presence of secondary tumors. For the dental profession, it is really challenging to diagnose oral precancer or oral cancer, particularly in its early stage of the disease. Early screening and advanced diagnostic tools play an important role in the early diagnosis and treatment planning of patients suffering from oral cancer. The diagnosis method usually includes histopathology and visualization adjuncts, such as toluidine blue, vizilite, etc. This study reviews the development of new upcoming tools for early detection, emphasis on the importance of role of dentist, and early detection of oral cancer.

Keywords: Early detection, Oral cancer, Screening.

How to cite this article: Singla S, Verma A, Goyal S, Singla I, Shetty A. Conventional and Advanced Diagnostic Tools in Oral Cancer with Emphasis on Role of Dentist and Early Detection. *J Postgrad Med Edu Res* 2017;51(3):128-133.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Worldwide, oral cancer accounts for 2 to 4% of all cancer diagnoses. Around 400,000 new cases of oral and pharynx cancer are reported every year. Depending upon the geographical region, prevalence of oral cancer varies as it represents approximately 10% of all cancer in Pakistan, whereas it accounts for approximately 45% in the Indian

subcontinent as a whole. Anatomic sites of oral cancer also vary with the geographic location.¹⁻³ Anatomic sites vary as the use of tobacco and related habits varies with geographic location. With change in geographic location, the habit also varies, which most importantly includes the percentage of tobacco used by the subjects.

Males are more commonly affected compared with females, with a ratio of 1.5:1. Older age group is the mostly affected age group, but now the trend is changing as the incidence rate of affected individuals less than 45 years of age has doubled from 1973. It may be due to change in lifestyle. Often, patients report to dentists or other specialists during the late stage of the disease; this leads to 45 and 32% 5-year survival rate after treatment of stages III and IV respectively. Although treatment for oral cancer is widely available, the mortality rate of overall disease is still high, i.e., 49%.⁴⁻⁶

ROLE OF DENTIST

Oral cavity and perioral region are most frequently examined by the dentist. So, dentists have an important role in detecting oral signs and symptoms of any disorder. Dentists even have an important role for the treatment and follow-up of any oral mucosal lesions. Dental examination should be done thoroughly. There should be primary goal to diagnose the lesion properly even in the early stage of the disorder as signs and symptoms of most of the diseases mimic each other. Diagnosis should be done before the metastases progress. After proper diagnosis, the dentist should decide whether abnormality requires further investigation. Diagnostic delay is an important factor that should be prevented by offering required investigation as prompt care of patient with oral cancer reduces the morbidity and mortality and offers a favorable prognosis of the disease to increase the quality of life. In scientific literature, a delay in dental treatment can be classified as (1) patients' delay, the period between the patient's first symptom and the first appointment with a health professional regarding the symptom described by the patient; and (2) service/professional delay, which corresponds to the period between the patient's first appointment with a health professional and definite diagnosis. Several studies indicate that dentists do not

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have sufficient knowledge regarding the etiology and diagnosis. Dentists should perform screening examination on every patient, especially in smokers and alcohol users every year regardless of their age.^{1,7-9}

IMPORTANCE OF EARLY DETECTION

The importance lies in the early detection in premalignant or early stage of the disease so that we can potentially limit the need for most invasive and disfiguring levels of the treatment to improve the quality of life of the patient. Early treatment means less invasive treatment that can prevent altered eating and swallowing patterns, salivary gland dysfunction, loss of structures, such as teeth, bone, and soft tissues including tongue, etc. In 2014, Singla et al¹⁰ concluded that at 60 Gy of radiation therapy, the unstimulated salivary flow rate becomes zero, which leads to dysgeusia, xerostomia, and oral mucositis. Early diagnosis reduces morbidity, improves the patient's long-term survival, and increases the quality of life. Sankaranarayanan et al¹¹ conducted a study in which they concluded there was reduction of mortality in smokers and drinkers of 43 and 22% respectively, after conducting oral cancer screening program.

CLASSIFICATION

Oral Examination

Oral cavity should be examined for oncological variations, which is simple, noninvasive, and within the skills of all dentists. Practitioner performs the examination usually with incandescent light, gauze, a mouth mirror, and magnification. There is a wide range of alterations seen in the oral cavity of patients suffering from oral precancer and cancer disorders. Alteration includes color change, i.e., red, white, or mixed; textural change, i.e., erosion, keratosis, granularity, loss of elasticity, fissuring or mixed; and additional signs include ulceration, induration, bone invasion, tooth mobility, and pain. In the later course of disease, lymphatic infiltration occurs.^{4,12}

Oral examination is not able to discriminate between the progressive, malignant, and those nonprogressive counterparts. Malignant changes may occur without any clinical changes in the oral cavity. In some studies, sensitivity and specificity vary between 58 and 94% and 76 and 98% respectively. So, other diagnostic tools should be used along with clinical oral examination to detect early changes.^{13,14}

Vital Staining

Toluidine Blue

It is a vital dye that has been used in the past for the detection of mucosal abnormalities of the cervix and the oral

cavity. It is an acidophilic metachromatic dye, which is used to stain acidic tissue component, sulfate, carboxylate, and phosphate radicals, such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), but not normal mucosa. As in malignancy, DNA synthesis increased and tumor cells are randomly arranged that helps in rapid dye penetration, which is the proposed mechanism of toluidine blue staining. First, the patient is asked to rinse with 1% acetic acid for 20 seconds followed by rinse with water twice for 20 seconds; after that the patient is asked to rinse the mouth with 5 to 10 cc. 1% toluidine blue solution followed by rinsing with 1% acetic acid solution (5 oz.) for about 1 minute again followed by water rinse. Dark blue stain is considered as positive for malignancy and light blue stain is considered positive for premalignant lesion unless proved by biopsy. Noninvasive, simple, inexpensive indication area for biopsy are the advantages whereas taste, stain remaining for 4 to 6 hours, and false-positive results are the disadvantages of toluidine blue.^{15,16} Overall, the sensitivity of toluidine blue staining for the detection of oral carcinomas varies between 78 and 100% and the specificity varies between 31 and 100%.¹⁷

Lugol's Iodine

Lugol's iodine is formed by 4 gm of potassium iodide and 2 gm of iodine in 100 cc of distilled water. First, the patient is asked to rinse with 1% acetic acid for 20 seconds, followed by water rinse for 20 seconds. Later, Lugol's iodine solution is applied to the suspicious lesion with cotton bud for 20 to 30 seconds and examined for retention of stain. Brown stain is considered as positive, while without retention it is considered as negative. Basis of this stain is glycogen content as normal epithelium has more glycogen content compared with malignant lesion. So, Lugol's iodine stains normal mucosa. Lugol's iodine staining even helps to know about differentiation of the lesions as poorly differentiated lesions without glycogen content do not have retention of Lugol's solution as compared with mildly differentiated lesions. Double staining technique which means use of Lugol's iodine along with toluidine chloride helps in differentiating the inflammatory lesion. Double staining technique is very useful to determine the biopsy site in suspicious cases.^{15,18,19}

Methylene Blue

It is a dark-green color odorless powder at room temperature, which generates blue solution when dissolved in water. Recently, it is suggested that methylene blue induces selective cancer cell apoptosis. It is cheaper and has low toxicity compared with toluidine blue, so can be used for oral screening on a large scale.^{20,21}

Rose Bengal Dye

Rose Bengal has been used to detect nonhealthy epithelial mucosa. Du et al²² conducted a study in which six lesions with normal clinical appearance were stained by Rose Bengal staining. So, the authors concluded that it can be used as a diagnostic procedure, but further research is required.

Cytopathological Examination

Oral Exfoliative Cytology

In this, a collecting device may be swab, spatula, or brush and is placed and rotated against the mucosal surface, and cells are collected. These cells should be spread on a glass slide to prepare a smear. These slides were examined by pathologist after fixation and staining. It can detect epithelial dysplasia, carcinoma, and atypical results. Navone et al²³ found this technique quite good for examination.

Micronucleus Assay with Acridine Orange

In this technique, alcohol-fixed smear is taken to distilled water, then smear is rinsed with water. After that, smear is rinsed in 1% acetic acid for few seconds, followed by two changes of distilled water for about 1 minute. Then, the smear will be stained with diluted acridine orange solution at pH 6.0 for 3 minutes, and staining smear will be rinsed with buffer at pH 6 for about 1 minute. After that, smear was differentiated with 0.1 M calcium chloride solution for ½ to 1 minute, then washed in phosphate buffer and mounted in the same. Frequency of micronuclei will be evaluated under fluorescent microscope. Singla et al²⁴ concluded that micronucleus is a non-invasive diagnostic tool to determine microscopic changes even during the normal clinical appearance.

Oral Brush Biopsy

Oral CDx is an oral biopsy system which is computer-assisted that collects transepithelial cellular samples. In this procedure, a special brush is placed on the lesion and rotated for 10 to 15 times till it produces reddening or hemorrhagic spots. Cell material which is obtained through brush is placed on dry slide, fixed, and sent for analysis. In the first published study in the United States, the brush biopsy reportedly detected correctly all cases of carcinoma.^{15,25,26}

Liquid-based Cytology

Liquid-based cytology (LBC) is introduced to eliminate many disadvantages of conventional exfoliate cytology. In LBC, sample is immersed in a tube containing preservative fluid, which fixes cells immediately. The fluid is centrifuged and makes high cellular confined zone on glass slide.²⁷

Tumor Markers and Biomarkers

These markers may be produced by cancer cells or by the host in response to cancerous agents. For detecting oral malignant lesions, cytokeratin markers can be used as these have altered expression, especially of buccal mucosa of oral cavity.¹⁹

Light-based Detection System

Chemiluminescence

It means emission of light by chemical reaction. Vizilite is most commonly used under chemiluminescence, which emits bluish-white light in a wavelength of 430 to 580 nm. In this technique, subject is asked to rinse with 1% acetic acid, which is done to remove the debris and to increase the visibility of nuclei due to mild cellular dehydration. The normal mucosa absorbs the light and appears blue, whereas abnormal mucosa appears aceto-white with brighter, sharper, more distant margins as it reflects the light. The reported sensitivity is 100% and specificity ranges from 0 to 14.2%.^{15,28} Recently, Vizilite system was modified to be used along with toluidine blue. So, MicroLux DL was introduced.

Tissue Fluorescence Imaging

In this technique oral mucosa is illuminated with intense blue light with a wavelength range from 400 to 460 nm. Normal mucosa appears pale green compared with abnormal mucosa, which appears darker due to altered structure and metabolism of epithelium and subepithelial stroma. MicroLux, Orascope, and Veloscope are included in tissue fluorescence imaging. Sensitivity ranges from 97 to 98% and specificity ranges from 94 to 100%.^{15,17}

Tissue Fluorescence Spectroscopy

In this technique, various excitation of wavelength produced by small optical fiber and a spectrograph is used to analyze oral mucosa. This technique has high sensitivity and specificity for the detection of malignant lesion from healthy mucosa. As optical fiber is small, only small mucosal area is scanned and therefore, not suitable for large mucosal lesion.^{15,17}

Identafi 3000

In this technique, anatomical imaging is used along with fluorescence, fiber optics, and confocal microscopy. As it is small in size, it is easily accessible in the unaccessible areas of the oral cavity. Besides detection, it is also used to examine tissue, i.e., detecting changes in angiogenesis with green amber light. Sensitivity of 82% and specificity of 87% are achieved to distinguish between neoplastic and nonneoplastic conditions of the oral cavity.²⁹

Optical Coherence Tomography

Optical coherence tomography is based on interferometer. In this, it is possible to get excellent spatial resolution and real-time imaging by using low-coherence broadband near infrared light source. It is a noninvasive, nonradiative diagnostic tool. In dysplastic conditions, cell size, shape, nucleus size, nucleus arrangement are more randomly distributed compared with normal mucosa. So, light scattering becomes stronger and more fluctuated. Cross-sectional images can be obtained of any mucosal area, whether it is normal or abnormal without biopsy. By using surface plasmon resonant gold nanoparticle, contrast images can be obtained.^{15,30}

Fluorescence Photography

Oral cancer, particularly oral squamous cell carcinoma, can be detected by fluorescence photography which is a noninvasive, rapid, simple, and reproducible method. Enlargement and progression of disease can be predicted by positivity of fluorescence.^{19,31}

Other Light-based Techniques

Contact endoscopy and endoscopic high-frequency ultrasound have been used in oral cavity. These new imaging techniques yield promising results in oral cavity, with sensitivity 91.3% and specificity 100%.³² Narrow band spectrum optical filters used to enhance visualization of mucosal pattern are used in narrow band imaging.³³ Further research is necessary to understand the diagnostic value of these technologies.

Saliva-based Diagnostic

Saliva can be used as diagnostic tool and it is an attractive, effective, noninvasive screening tool for the detection of oral cancer. It is based on the fact that salivary composition is altered in patients with oral cancer. Salivary diagnosis depends upon the quantitative analysis of specific salivary macromolecules, enzymes, cytokines, growth factors, metalloproteases, endothelin, telomerase, cytokeratins, messenger RNA, and DNA transcripts. Total sugar, protein-bound sialic acid, free sialic acid, sodium, calcium, immunoglobulin G, albumin, lactate dehydrogenase levels are much higher in saliva of oral cancer patients as compared with normal patients. Oxidative stress-related salivary parameters, epithelial tumor markers cytokeratin-19 fragments in serum (CYFRA 21-1), tissue polypeptide-specific antigen, RNA transcripts [insulin-like growth factor, matrix metalloproteinase (MMP)-2, MMP-9, interleukin-8 and 1B] levels are significantly altered in oral cancer patients. Studies have shown that saliva of oral cancer patients contains

statherin, histatin 3, proline-rich proteins, spermidine N1 acetyltransferase, β -actin, and glyceraldehyde-3-phosphate dehydrogenase. Levels of salivary soluble CD44 are significantly increased in oral cancer patients, but there is no correlation found between the level of CD44 and stage of the tumor. More studies are required to investigate further as, to date, saliva-based testing has not been incorporated into a commercial product. Presently, it is more costly and not easily accessible as it is done in some higher specific labs only.^{16,34}

Microfluidics

It is also referred as lab-on-a-chip or micro-total-analysis systems. Microfluidics accept saliva and are suitable for handling living cells. Dysplastic cells can be detected in this chip. Stage and type of the cancer from which patient is suffering can be detected by this technology. It is suitable for use in clinics or operating room.³⁰

Deoxyribonucleic Acid Ploidy

The nuclear DNA content is measured by DNA ploidy. If chromosomes become detached or are not uniformly distributed to the daughter cell, then chromosomal segregation is termed as unbalanced during mitosis. This is called aneuploidy and observed in dysplastic lesions. In this technique, cytological samples are compared with a reference group after staining and computer-assisted analysis is done for genomic instability. It is a noninvasive procedure with high specificity.^{15,35}

Biopsy

For diagnosis, incisional and punch biopsies are more commonly used. Histopathological examination determines the nature of dysplasia, i.e., mild, moderate, or severe, as it is the gold standard for the diagnosis of potentially malignant disorders. For the pathologist, adequate sample size is required to determine the proper diagnosis. If the sample is not adequate, then there are more chances for discrepancy in the report.

Imaging Modalities

Diffuse Reflectance Imaging

When light enters the tissue it may be elastically scattered, inelastically scattered, or absorbed. The property of light depends on the tissue morphology, such as nuclear size, distribution, epithelial thickness, collagen content, amount of oxy and deoxy hemoglobin present in the exposed tissue. All these content varies during transformation from normal tissue to malignant one. The resulting signal of the light after interaction with tissue is detected by fibers and fed into a spectrometer interfaced

with a computer. Diffuse reflectance spectroscopy is a noninvasive optical tool for early detection of malignant changes in the tissue of the oral mucosa.^{16,30}

Fluorescence Endoscopy

Fluorescence endoscopy is much more accurate and specific than white light endoscopy. It involves the use of photosensitizing drug to visualize lesion through tissue fluorescence. Most commonly used photosensitizing drugs used in endoscopy are 5-aminolevulinic acid and hypericin. Malignant lesions have higher uptake of drug compared with normal mucosa.³⁰

Positron Emission Tomography Scan

Early diagnosis and staging of the tumor can be done by positron emission tomography. Fluorodeoxyglucose is injected and scan is done. Even early involvement of lymph node can be detected. So, early diagnosis of oral cancer can be done by this technique.

Others

Polymerase Chain Reaction-based Diagnostic Tools

The polymerase chain reaction can be used in the study of cancer and provide the information on the genetic etiology of neoplasia, as it can detect mutations in oncogenes and tumor suppressor genes. There is difficulty in the interpretation of the results due to contamination, which is one of the major disadvantages other than being cost-effective, selectivity, availability, etc.^{19,31}

CONCLUSION

Potentially malignant disorders pose an important threat to the overall survival of an individual. The ability to identify lesions and to predict which lesions will undergo malignant transformation would facilitate early diagnosis and subsequent disease management tailored to the individual patient. The ultimate goals are to reduce both mortality and morbidity, and to improve patients' quality of life. Detection tools are becoming increasingly accurate and less invasive as studies continue to be published in order to determine the sensitivity and specificity of each detection mechanism. Integration of the adjuncts and tests discussed here can help uncover hidden lesions before they have the chance to progress into malignancy. Hence, all practitioners should be aware of the latest advancements and should try to incorporate them whenever feasible.

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