Review Article

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# Newer Diagnostic Tools for early detection of Oral Cancer: A Review

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## ABSTRACT

**Background**: Oral cancer comprises of genetic damage over time. The older cancer diagnostic aids had less specificity, were time-consuming, and produced inter-observer bias. Advancements in the technology and curative methodologies in the past few years have helped to diagnose and treat this disease at an early stage. Advances in molecular and genetic biology have helped us to better understand the complexity amongst genetic, transcriptional, and translational alterations in human cancers. This review provides a summary of all the diagnostic modalities that were used earlier and the newer more advanced techniques with merits and demerits of each technique described briefly.

**Keywords:** Genetic biology, Molecular biology Oral cancer.

## INTRODUCTION

Oral Cancer is the 3rd most common type of cancer occurring in developing countries with approximately 80000 new cases annually.It ranks 6thin the overall incidence for the 10 most common cancer sites worldwide and 3rdin the developing countries. [1][2]The rise of newer treatment modalities have not deterred the mortality rate; treated cases having a 5 year survivability of around 50%.[1][11]One of the most common hindrances for this is the late detection of such lesions, which dramatically affects the success ratio. Early diagnosis results in a boost to curing the condition with minimum damage to form and function.[10]It also serves to recognize people at a higher risk for cancer as conservative treatment can be carries out with a better recovery rate and improved quality of life. Suspected patients should be more intensively scrutinised and thorough treatment protocols are to be carried out. The current inclination in diagnosis is based in the areas of molecular biology, and advanced equipments will make us progress in the field of disease management.Conventional diagnostic techniques, besides being time consuming, also have less specificity and inter-observer bias.[8] The advancement of scientific technology by leaps and bounds in the past few years have resulted in multiple new and advanced diagnostic methods. This review aims to cover such techniques in a comprehensive manner.

### Aim and Objectives

This review aims to spread awareness regarding the newer diagnostic tools in detection of oral cancers that may have a significant impact on clinical practice. By this study, we will come to know the shortcomings of conventional techniques associated with late detection of cancer and gain an in depth knowledge of better and technologically advanced procedures.

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## MATERIAL AND METHODS

Articles were searched for on both mediums – electronic search and hard copy search. Electronic searches were based on the title using keywords – "Cancer diagnostic techniques", "newer advancement" and "conventional cancer detection methods". Database searches were made across multiple indexing platforms like PubMed, IndexCopernicus, Scopus, Google Scholar, Medline, J Gate and ADA. Hard copy searches were done amongst relevant articles published in JOMS, IJOMS and BJOMS.

Articles to be included had to satisfy the following criteria –

- Published within last 5 years
- Those which have applied the procedures clinically
- Having thorough description of the application and rationale of such investigations

Similarly those that were excluded from this review included –

- Articles not providing detailed description of the procedures
- Articles which provide a biased opinion of the techniques
- Articles which do not provide a clinical application of the technique

A total of 34 articles were selected by means of electronic search(27) and hard copysearch(7). The criteria for selection was on the basis of title; the studies containing relevant materials in their titles were taken up. Following this, a more in depth study was done on the abstracts of these chosen studies which led to the elimination of 16 articles.

A total of 18 studies went to the final round of selection, which consisted of a detailed and thorough analysis of the full text matter. 8 articles did not meet with the inclusion criteria guidelines and so a final number of 10 articles were selected for this particular review.

The chosen articles had a chronological distribution as given below –

- 2015 – 3
- 2016 – 4
- 2017 – 2
- 2018 – 1

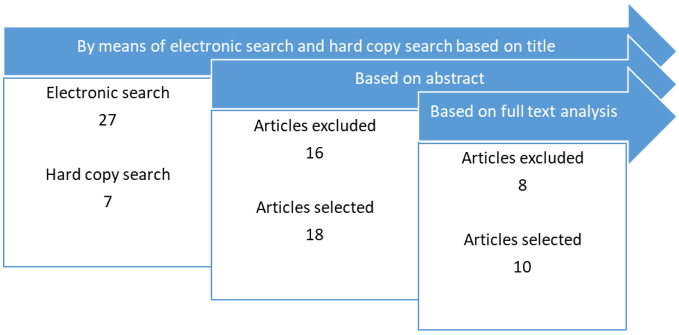


Fig 1: Flowchart depicting the selection procedure of the chosen studies

### Conventional Techniques

### Vital Staining

Toluidine blue, an acidophilic metachromatic dye is used as a vital stain, for the recognition of dysplastic cases and early oral squamous cell carcinoma (OSCC). These cells contain greater assessable amount of nucleic acids when compared to normal tissues. Additionally, intracellular canals of the cancerous epithelium are larger than ordinary epithelium leading to greater penetration of the dye.

### Oral Brush Biopsy

Oral brush biopsy is based on the concept of exfoliative cytology which provides cytological evaluation of cellular dysplastic changes. The procedure is rapidly conducted chair side that is cost-effective and perhaps the best approach for the initial evaluation and diagnosis of oral diseases. It permits narrowing down of cases for biopsy and localize the optimal site for brushing alesion. Traditional oral brush biopsy combined with the application of toluidine blue plays an important role in pin pointing suspected mucosalareas.

### Liquid Based Cytology

It refers to a method of preparing and processing smears. A sample of cells from the lesion is taken and placed into a liquid solution (agarose, polyethylene glycol, poly-l-lysine, alcohol). The material collected in the liquid fixative preserves the cells. In CLBC, a centrifugation machine helps to remove impurities like excess blood, mucous, and inflammatory cells. The cells are then stained and examined under the microscope.

**Histopathology**

This type of technique evolved from an era of diagnosis based on haematoxylin and eosin stained slides. There are grading systems present to predict tumour aggressiveness and the pathologist's report determines the surgical and pharmacological decisions. For many, this method is considered the gold standard for diagnosis and staging of many diseases.

**Immunohistochemistry**

In this technique, antigen-antibody interactions are usedfor identifying cellular or tissue constituents.Directlabelling of the antibody or a secondary labelling method are used to identifythe site of antibody binding. Conventionally used special and enzyme staining procedures identify only a limited number of enzymes,proteins, and tissue structures and IHC has a definite edge over them due to its higher spectrum of identification.

**Polymerase Chain Reaction**

PCR in molecular biology is used to amplify a single copy or a few copies of a piece of DNA across several orders of amplitude, generating millions of copies of a particular DNA sequence. It is based on thermal cycling - consisting of cycles of recurrent heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. It uses multiple genomic targets likeenzymes, cytokines, growth factors, metalloproteinases, endothelin, telomerase, cytokeratins, mRNAs, and DNA transcripts.

**Microarray Technology**

It is associated with the quantitative study of mRNA. It is a compositeminiature composition that is a two-dimensional alignment on a solid substrate that evaluates large amounts of biological material using high-throughput screening miniaturized, multiplexed and parallel processing and detection methods.The expression levels of thousands of genes are assessed which provides a unique sketch of panel of genes and the levels of change in a given disease.

**Newer Techniques**

**Nanodiagnostics**

This technique uses the application of nanobiotechnology that takes its reference from pharmacogenetics, pharmacogenomics, and pharmacoproteomics information. It involves the application of nanoparticles; the use of manufactured nanorobots - quantum dots (QDs), gold nanoparticles, and cantilevers to make repairs at the cellular level. Various biomarkers are analysed through this procedure. Furthermore, the management of cancer through personalized medicine is made possible through this technique.

**Velscopy**

The notion of tissue autofluorescence was used in gauging of cancerous lesions in the lung, uterine cervix, and skin previously. This technique has been modified and is being used for oral cavity. The Velscope uses a blue light that stimulates a green fluorescence that reaches its peak intensity at approximately 436 nm. This method is based on the different cellular responses of dysplastic and normal cells to the light, with the former giving a green fluorescence.

**Laser Induced Florescence**

LIF uses spectroscopic techniques, whereinlaser light is directed towards an atom or molecule which in turn gets excited to a higher energy level and then returns back to its original state resulting in the spontaneous emission of light. This method can be used to study the structure of molecules and detection of selective species. The emitted wavelength is higher than the excitation wavelength and its wave form is usually in the minute field of a few nanoseconds to microseconds. Filtered photodiodes or a photomultiplier tube are used to record such emissions. Fluorophores such as elastin, collagen, and nicotinamide adenine dinucleotide hydrogen (NADH) are responsible for such behaviour.

### Ratio Imaging

Analysis of ion concentration change in living cells, such as calcium ion and pH, is the main working principle of this technique. Cameras that have the capacity for capturing multiple spectrum images are required, which have features like high stability and high quantitative precision. Comparison is made between a photochemical or metabolic end-product of the intracellular compound; one which is increased in disease state, and another that is decreased in the same diseased state. Ratiometric fluorescent probes are used which can study, record and evaluate these readings at the single cell level.

### Elastic Scattering Spectroscopy

Elastic scattering spectroscopy requires light to be refracted off of the intracellular components that generates a spectrum dependent on wavelength. This spectrum is collected and analysed by a spectrometer and changes are identified by using therefractive indices of the cellular componentssuch as the chromatin, nucleus, sub-cellular organelles, structural proteins, lipids, and erythrocytes. The light refracted is around330 to 850 nm which falls under the visible light spectrum and near the ultraviolet spectrum.

### Raman Spectroscopy

Raman Spectroscopy is a spectroscopic technique based on energy levels of tissue molecules by definite frequencies, above and below the incident photon that emerges out after interacting with the tissue.This method is used to observe vibrational, rotational, and other low-frequency modes in a system which provides a fingerprint by which molecules can be identified. Thus, it can detect changes in the biomolecules of the tissue.

### Multiphoton Excited Fluorescence

Multiphoton excited fluorescence is a method that allows imaging of living tissues up to a depth of about one millimetre. This is made possible through the use of advanced optical method that uses high-resolution fluorescence imaging. Hence, different changes in fluorescence intensity associated with cancer development even at the cellular level can be studied. 3D images of endogenous tissue fluorescence are obtained due to thehigh-resolution capability equipment that can effectively distinguish between normal, precancerous, and cancerous epithelial tissues.

Table 1: Conventional Techniques.

### Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy provides detailed information about the dynamics, structure, chemical environment and reaction state of molecules. The electronic structure of various molecules can be analysed by assessing the intramolecular magnetic field that causes changesin the resonance frequency. This allows the study of atoms which in turn helps in gene transcription and signal transduction.

## DISCUSSION

The field of oral cancer is vast and complex. Diagnosis and treatment planning of such lesions is very essential. Advancement of technology has made a multitude of newer techniques available to us which provide a more accurate and conclusive view of the disease.

K. ShwethaNambiar et al discussed the perplexing problems associated with the detection, diagnosis and management of oral diseases. Advancements in this field will undoubtedly improve the detection rates and time period involved to achieve that stage. Thus higher quality understanding of the cellular level processes will enable in better scrutiny and minimum impairment and deformity. [1]

Roopa et al stated that there is a definite lack of awarenessabout the signs and symptoms of oral cancer amongst the general population. A major struggle is the education of the populace regarding the mortality and morbidity from late stage detection and treatment. Thus, invention of new technologies with faster detection and greater specificity can help lead to early detection and minimize damage. [6]

| Technique | Methodology | Merit | Demerit |
|---|---|---|---|
| Vital staining | -Toluidine blue, an acidophilic metachromatic dye<br>-Stains greater amount of nucleic acids of dysplastic cells | -Mass scale biopsy<br>-Presence of cancer detected | -Non specific<br>-Margins cannot be clearly identified |
| Oral brush biopsy | -Exfoliative cytology | -Chair side<br>-Cost effective<br>-Mass scale | -Non specific<br>-Inadequate sampling<br>-False negative |
| Liquid based cytology | -Smearing fixation<br>-Microscopic examination | -Uniform smear<br>-Sensitive | -Less specific<br>-Interpretation bias |
| Centrifuged LBC | -Similar to LBC but slide is centrifuged | -Better removal of blood and mucous | -Technique sensitive |
| Histopathology | -Microscopic examination | -Gold standard<br>-Reliable and inexpensive | -Technique sensitive<br>-Error prone<br>-Time consuming<br>-Inter observer bias |
| Immunohistochemistry | -Labelling in antigen-antibody | -Sensitive to microbes<br>-Archival material can be used | -Technique sensitive |
| Polymerase chain reaction | -DNA cloning for sequencing | -Specific<br>-Diagnosis of hereditary diseases | -Fluctuates results by contamination |
| Microarray technology | -Quantitative study of mRNA | -Gene detection in one go | -Time consuming and labour intensive<br>-Mandates DNA preservation |

**Table 2: Newer Techniques**

| Technique | Methodology | Merit | Demerit |
|---|---|---|---|
| Nanodiagnostics | -Manufactured nanorobots make repairs at cellular level<br>-discovery of biomarkers | -Applicable for small samples<br>-sensitive, high speed, and flexible biological tests | -Nanoparticles are hazardous to health<br>-legal/ethical concerns |
| Velscopy | -Blue light with peak intensity at 436nm<br>-Green fluorescence | -Mass screening<br>-Easy to use<br>-Identification of boundaries | -Initial cost |
| Laser-induced fluorescence | -Atom or molecule is excited to a higher energy level by the absorption of laser light and then followed by spontaneous emission of light | -Non-invasive<br>-sensitive<br>-accommodates laser imaging<br>-detects micro changes within cells | -Cumbersome technique<br>-potential photochemical effects |
| Ratio imaging | -Analyze ion concentration change in living cells such as calcium ion and ph<br>-Cameras with high stability and high quantitative precision for capturing multiple spectrum images | -Non-invasive<br>-prefers the single focal plane<br>-improved optics and imaging | -Cumbersome technique |
| Elastic scattering spectroscopy | -Cellular and sub-cellular changes are identified using the refractive indices of the cellular components<br>-The resultant scattered light is collected and analyzed by a spectrometer, and a spectrum is generated | -Sensitive and optically guided biopsy<br>-detects micro changes within tissues | -Fails to detect tumor margin<br>-time-consuming |

**Table 3: Newer Techniques**

| Technique | Methodology | Merit | Demerit |
|---|---|---|---|
| Raman spectroscopy | -Unique optical diagnostic technique that can detect changes in the biomolecules of the tissue<br>-Energy levels of tissue molecules by definite frequencies, above and below the incident photon that emerges out after interacting with the tissue | -Highly specific<br>-facilitates remote analysis<br>-Raman spectra can be collected from a very small volume (<1 μm in diameter)<br>-avoids sample preparation<br>-waterproof and non-damaging | -Sensitive and optimized instrumentation<br>-artifacts hinder the Raman spectrum |
| Multiphoton excited fluorescence | -Excitation wavelength here is shorter than the emission wavelength<br>-Wavelengths of the two exciting photons are longer than the wavelength of the resulting emitted light | -Suited for imaging in optically thick specimens<br>-higher axial resolution<br>-reduced photo-bleaching of marker dyes<br>-increased cell viability | -Photo and thermal damage |
| Nuclear magnetic resonance spectroscopy | -Intramolecular magnetic field around an atom in a molecule changes the resonance frequency | -Characterizes micro samples<br>-exhaustive information about a molecule's structure<br>-explores structural information of unknown compounds<br>-free of biologic hazard | -Expensive<br>-Mandates consistent signal-to-noise level |

Gaurav Sharma reported that the WHO has stated that early detection and proper prevention are the hallmarks in fighting the onslaught of cancer. Limitations of current and prevailing techniques include low sample volumes, errors and inter observer bias. Newer methods do away with these shortcomings but still further research is required in clinical practice and long term studies need to be performed. [7]

K. N. Shwetha and associates pointed out that requirement of fresh samples, along with need for freezing of samples and high quality sample banks cause delay in detection along with multiple sampling and processing errors. However, newer technologies circumvent this issue and faster and more accurate diagnosis can be performed.[2]

## CONCLUSION

A major deterrent in the early detection of oral cancer is the lack of awareness and the time consuming conventional diagnostic techniques along with the errors and bias associated with those same techniques. Newer diagnostic methods completely bypass these deficiencies and proper quick, reliable and accurate readings that allow for faster treatment plans and reduced morbidity to the patient. Further research is needed to make such technologies more available and cost effective for the masses. The cost of setting up and maintaining the infrastructure is definitely of a large volume but when compared to the physical and psychological effects on the patient and family, due to the post-surgical outcomes, such costs can be deemed imperative for the ultimate goal of improving the quality of life.

## CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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