Review Article

International Journal of Research in Pharmaceutical Sciences

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Diagnostic Aids and Techniques of Oral Cancer- An Updated Review

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Article History:

Received on: 23 Oct 2022
Revised on: 24 Nov 2022
Accepted on: 28 Nov 2022

Keywords:

oral cancer,
cytology,
biopsy,
oral scan

Abstract

Oral cancer is the most prevalent and lethal type with a four in every 3000 incidence rate worldwide and a 3% survival rate. Oral squamous cell carcinoma (OSCC), which is multifactorial, is brought on by genetic and epigenetic instability. Oral potentially malignant disorders (OPMDs) are precursor lesions that often precede oral cancer, and their early diagnosis is advantageous for patients since it may lengthen their productive longevity. Currently, oral cancer screening, early identification, and its pre-invasive intraepithelial phases are still largely focused on visual inspection of the mouth. These methods are subjective and early lesions can be easily missed. Due to a lack of early identification, oral cancer's five-year survival rate is still low. Recently, the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) emphasised that by developing efficient cancer control and screening strategies, we can prevent a third of the 15 million cancer cases that are expected to occur in the future and better manage a second third. Oral cancer screening aids and techniques have witnessed a lot of advancements recently. This article reviews the current diagnostic methods and instruments for detecting oral cancer, which include the oral CDx brush, Velscope, Chemiluminescence, DNA ploidy, microarray technology, colposcopy, and oral scan. It also adds on the molecular (genetic and epigenetic alterations) & decoding of the oral carcinogenesis genomics data.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v14i1.4177

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INTRODUCTION

Due to its high rates of morbidity and mortality, oral cancer—a disease that affects everyone—has developed a reputation for being difficult to treat [1]. Approximately 643 000 new cases of head and neck cancer (H&N cancer), which includes all oral, laryngeal, and pharyngeal sites, are diagnosed each year [2]. The greater morbidity linked to this fatal disease is attributable to the disease's delayed diagnosis and advanced stage presentation [1]. Preventing oral cancer and detecting it early are two key goals in reducing its prevalence worldwide, according to the World Health Organization [2].

Diagnostic Aids, Techniques & Recent Advances

Cytopathologic Studies

Brush biopsy

It is sometimes referred to as the OralCDx Brush Test system, which is a technique for obtaining a sample from the lesion of the mucosa of the trans-epithelial cell that represents the basal, parabasal, and superficial layers of the epithelium. Due to its low-risk

clinical characteristics, this test was created primarily to look at abnormalities in the mucosa that usually do not necessarily require a biopsy. Samples of epithelial cells are smeared on a glass slide, which is then prepared for a modified form of the Papanicolaou test, and it is assessed via a microscope. A brush that is specifically made is used as the device without causing any laceration for collecting cells of the epithelium [2].

Liquid based cytology with Oral CDx brush

A dedicated oral tool (such as a CDx brush) has never been used in any liquid-based cytology research in the oral cavity; instead, sample collection has always been done using cervical or dermatological techniques. Inadequate results are anticipated since cervical brushes are not stiff [3]. An accuracy of 92.3 percent was found in a study by Mozafari et al with oral CDx brush, this may help to improve sensitivity and alleviate the issue of false negative and subpar outcomes [4, 5].

Light Based System

Chemluminescence (Reflective tissue fluorescence)

The chemiluminescence method involves rinsing the mouth with one percent acetic acid, thereby helping to clear away debris and making epithelial cell nuclei more visible because of minor cellular dehydration [6].The aberrant tissue will reflect the blue-white illumination, allowing the occult lesion (aceto-white & reflect light) to be distinguished from healthy mucosa(blue) [7].

Tissue fluorescence imaging (Velscope System)

A strong blue excitation light (400-460 nm) is used in this technique to illuminate the oral mucosa. This causes the aberrant tissue to glow as a result of altered epithelial and subepithelial stromal structure and metabolism [8]. Healthy mucosa displays an autofluorescence that is pale green, whereas the aberrant tissue tends to appear darker when compared to the normal tissue surrounding it [9]. According to case studies, the veloscope (Figure 1) has a high sensitivity (98–100%) and selectivity (3–100%) to identify the areas that have beyond changes of dysplasia and cancers that have expanded lesions that are clinically visible [7].

Tissue fluorescence spectroscopy

A spectrograph is used in this technique to gather, record and analyse the spectrum of the tissue's fluorescence that is reflected. A tiny optical fibre produces a variety of wavelengths that are excitation [10]. Technology distinguishes malignant tumours from healthy oral mucosa with accuracy

Figure 1: Velscope& Detection of oral cancer by Velscope

and success. Because the optical fibre can only assess a small area of the mucosa, spectroscopy is only used to assess well-defined small mucosal lesions that have already been diagnosed through clinical inspection to determine whether they are benign or (pre) malignant [11]. This technique is therefore unsuitable to detect lesions that are new or to assess larger lesions [8].

Oral scan

An optical imaging multimodal tool called Oral Scan is used to find (pre-)cancerous lesions in the oral cavity as early as possible. The oral scan (Figure 2) system functions according to the diffuse reflectance and tissue autofluorescence theories, in which light is repeatedly absorbed and scattered before emerging from the tissue surface. Optical signals coming from tumour tissues are altered by the biochemical and morphological changes that occur during the carcinogenesis process.

Before taking a biopsy, the doctor can use OralScan to visualise and distinguish between healthy and potentially cancerous oral cavity regions. It is not an invasive procedure, the procedure is done in vivo, it images a very large field by using a cloud based machine learning algorithm to obtain results on the status of tissue and applies oxygenated hemoglobin (HbO2) absorption maps to guide the biopsy. All of these Oral Scans' diagnostic applications can be very beneficial in cancer diagnosis [12].

DNA Ploidy

The amount of nuclear DNA is gauged by DNA ploidy. Feulgen dye-stained cytological samples are collated into an instance set of cells and analysed via computer to spot variations in cellular DNA concentration. Genomic instability contributes to cancer growth, and aberrant DNA content distinguishes dysplastic lesions from other cancers [5].

Figure 2: Oral Scan

Microarray technology

An array of DNA spots on a solid surface is represented by a DNA microarray. Utilizing this method, researchers may examine how different genes are expressed in diverse cancer types. A stand-in marker for this is messenger RNA (mRNA). In this approach, multitudes of oligonucleotides or fragments of DNA get covalently bonded onto a chip (solid surface) and arranged in rows and columns in a predefined sequence in either a 2D or 3D configuration. Reverse transcription and labelling of the sample RNA would enable the identification and quantification of particular transcripts. Restriction endonucleases are used in the microarray approach to cut unknown DNA segments, allowing fluorescent markers to respond to chip probes of DNA. The probes cohere with the DNA fragments. Fluorescence emission enables a recognition of the target DNA pieces. The examination of numerous molecular markers from a sample of one patient is made easier by gene expression arrays. Microarrays are used in OSCC to identify single nucleotide polymorphisms (SNPs), gene mutations, cancer biomarkers, and genes involved in drug discovery and chemoresistance [1].

Next-generation sequencing

Sanger sequencing was used to sequence the first piece of DNA in 1977. Second-generation sequencing was first launched in 2005. DNA extraction with the rapid gathering of extensive sequencing data is made possible by NGS technologies. Furthermore, NGS techniques provide an important understanding of genetic paths, allowing us to comprehend the onset and progression of the disease. Various NGS techniques can be used for DNA sequencing, whole genome characterisation, coding genome analysis, copy number checks, translocation detection, and mRNA abundance assessment. The Illumina/Solexa Genome Analyzer, Roche/454 FLX, the Helixos Heliscope TM, Life Technologies Ion Torrent, and other NGS systems are commercially available. Third-generation sequencing (TGS) has recently been made available using one molecule. NGS systems have made it possible for us to comprehend the numerous genomic changes found in OSCC [1].

Colposcopy (direct microscopy)

Colposcopy is a well-known procedure of diagnosis that is implemented to examine vaginal, vulvar, and cervix tissues while illuminating the area of interest with a magnified view. When using a portable video camera attached to a colposcopy device, it is possible to observe three-dimensional images of the tissue surfaces being inspected on a television monitor. white or yellow light, which is unfiltered blurs the distinction between arterioles and surrounding tissue, so the colposcope is equipped with a blue or green filter to authorise the evaluation of changes in vascularity and colour quality. The ideal working distance for the microscope's focal length is 200 mm. According to a study, colposcopy done on premalignant lesions of the mouth was accurate in identifying oral mucosal abnormalities in the range of 70% to 98 percent [10].

Salivary Biomarkers

Over a hundred possible biomarkers of the oral cavity have been identified in the English literature, mostly based on comparisons between the amounts found in patients with the disease and those found in healthy individuals serving as controls. A number of salivary proteins have been studied, including a-amylase, interleukin 8, tumour necrosis factor-a, Statherin, CA 125, Endothelin-1, CD44, Catalase, Cyclin D1, and CEA. The few difficulties in using this technique include the lack of valuation for the technique of collecting samples of saliva [10].

Cell and tissue markers

Epithelial growth factor (EGF), Cyclins, AgNOR, bcl2, and telomerase have all been employed as tumour growth markers [23]. Four hypoxia biomarkers—GLUT-1, carbonic anhydrase IX, hypoxia inducible factor la, and erythropoietin receptor—as well as three angiogenic biomarkers—CD105 and Eph receptor tyrosine kinases (Ephs), vascular EGF, have been found as biomarkers. Tumour suppression markers and an anti-tumor response include retinoblastoma protein, p53, and cyclin-dependent kinase inhibitors. The matrix metalloproteins are proteases that are frequently evaluated in various research, and they are commonly expressed by invasive tumours and adjacent stroma. Desmoplakin, Integrins, and cathepsins have all been identified as

indicators of tumour invasion. Investigations have been done on cytokeratins, filaggrin, involucrin, and glutathione S-transferase [10].

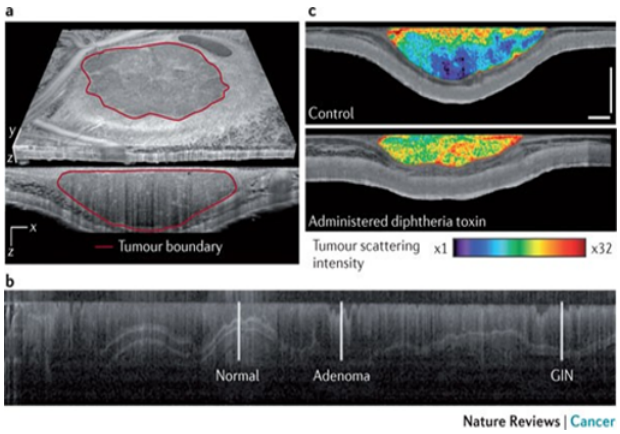

Figure 3: Optical coherence tomography revealing tumor

Elastography

One important factor used to distinguish an enlargement that is inflammatory and malignant is the hardness (elasticity) of the lymph nodes. Elastography evaluates the cellular structure's compliance behaviours. Tissue hardness can be calculated by measuring the displacement or strain that tissue compression causes in the tissue's structural elements. The elastography images are compared before and after cervical lymph node compression [10].

Surface enhanced Raman spectroscopy

This method provides a precise, highly accurate acquisition of the structure of the molecular tissue because of the unique way that biological molecules interact with photons. Lipid, nucleic acid, and protein spectral characteristics serve as accurate Raman indicators to distinguish between cancerous and healthy oral mucosal tissue. Raman spectroscopy contributes knowledge that is comparable to or even superior to established methods in oral carcinogenesis. The drawbacks include a lack of spatial information, intensive processing, expensive equipment requirements, randomness, nonimaging, and complex algorithms to separate different tissue classifications [10].

Positron Emission Tomography

The fluorodeoxyglucose-positron emission tomography (FDG-PET) test exhibits excellent prognostic precision and importance in characterising lymphatic status, aiding in the analysis along with prompt identification of cancer of the oral cavity. PET or computed tomography (CT) could detect as well as differentiate persistent or recurrent neoplasias from surgical or radiation-induced alterations because malignant cells contain an increased amount of FDG for a lot of time when compared to infectious and inflamed structures. According to recent studies, PET/CT was highly accurate (> 90%) at finding the recurrent tumour [10].

Optical coherence tomography

A minimally invasive tomographic imaging technique is optical coherence tomography (OCT). The method creates a cross-sectional architectural representation of the tissue using subsurface reflections to identify areas of inflammation, dysplasia, and malignancy. The oral mucosa can be imaged using OCT technology with insertion into the tissue up to one to two mm deep [4]. Optical coherence tomography involves capturing below the surface pictures to provide a comprehensive cross-sectional picture (Figure 3). The difference between in-vivo images of malignant lesions of the oral cavity in a hamster is enhanced by the multimedia dispersion of polyethylene glycol that is connected to gold nanoparticles attached to antibodies. A recent pilot study involving 27 cancer patients revealed the viability of using optical coherence tomography to find structural alterations in malignant molecules [10].

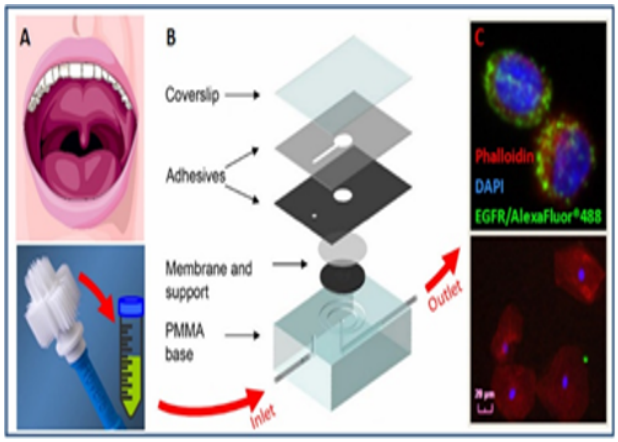

Figure 4: Bio nano-chip

Highly accurate, yet user friendly, inexpensive and non-invasive technology for detecting oral cancer in resource-constrained clinical settings has been developed by Scientists at IIT Kharagpur (Indian institute of technology) which is based on OCT.

This diagnostic device is a portable and easy to operate blood perfusion imager (BPI) along with a miniature far-infrared (FIR) camera and a humidity sensor, which have been controlled via electronic as well interfaced along merge with physics-based and a software engine that is driven by data.

The actual gadget is made up of a probing unit to screen and a processing unit to gather information

about blood perfusion along with diagnosing diseases. The sensor housing and holder that make up the probing unit keep the sensors in a stable environment while minimising the effects of breathing. The utility of the holder is to guide sensor housing towards the site of measurement. Sensor housing comprises a digital humidity sensor that is completely computed for detecting the ambient temperature and relative humidity in the oral cavity, as well as an on-chip long-wave infrared (IR) camera for measuring the temperature of the tissue. Using additional signal-processing electronics, the IR camera sensor array converts into temperature values, the radiometric values alongside thermal sensitivity 50 mK (milli Kelvin). The imaging occurs at a rate of 8.7 Hz, capturing spectral illumination in the wavelength range of 8 to 14 m (micrometre).

Bio-Nanochip

A novel bio-nanochip (BNC) (Figure 4) sensor was recently documented. It is quite a quick cytology test of the oral cavity that combines the benefit of cytological morphometric analysis alongside the quantification of neoplastic biomarkers. Microfluidics technology, sometimes known as "lab-on-a-chip," is generally defined as the adaptation, miniaturisation, fusion, and automation of analytical laboratory techniques into a single chip. The BNC sensor used membrane-related cell proteins, which are particularly prevalent in the cellular membrane structure of malignant cells, to identify cancerous cells [10].

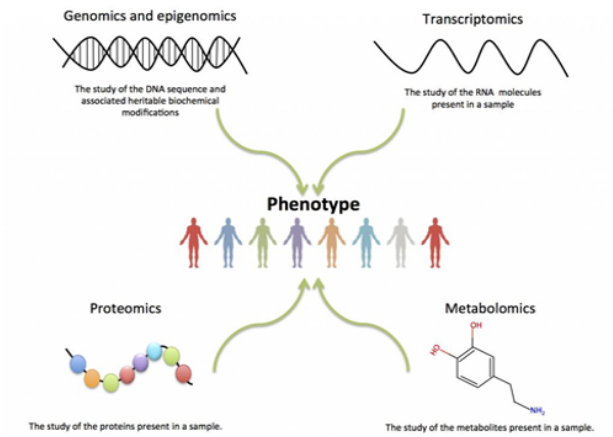

Figure 5: Genomics, proteomics, transcriptomics

PCR-Based diagnostic aids

Polymerase chain reaction (PCR) can be used to examine and diagnose infectious diseases and cancers linked to microbes. PCR is a crucial method for detecting mutations occurring in cancer-related oncogenes (such as K-ras and N-ras), tumour suppressing genes (such as p53 and p16), and other genes. The PCR technology has expanded the scope and diagnostic technique sensitivity, however, there is still a significant downside, due to the possibility that contamination and amplification artefacts could make it difficult to understand the required results. PCR, reverse transcriptase PCR (RT-PCR), and various molecular techniques have made it possible to diagnose and predict the prognosis in additional lesions, like chronic myelogenous leukaemia [11].

"Omics" in oral cancer

By decoding oral carcinogenesis, genomics data considerably improve our knowledge and understanding, which in turn aids in the development of targeted treatments and prognosis prediction. In order to finally improve the lives of OSCC patients, high-outturn technology is being applied to give insight into the underlying molecular pathways (genetic and epigenetic alterations) in OSCC [1].

Genomics

Genomic profiling (Fig 5) of OSCC is essential taking into account the inter-tumor and intra-tumor heterogeneity. Various alterations in the chromosome are reported in OSCC for instance, loss at 3p and WISP1 genes. Recently, core dysregulated pathway and target that is actionable are identified, which might dictate the use of more involved and affordable sequencing panels [1].

Transcriptomics

By using the high outturn technique, transcriptomics concerns to study of transcriptome, that is the absolute set of RNA transcripts made by the genome under specified conditions [1].

Proteomics

Proteomics (Figure 5) is the study of the full range of proteins expressed in an animal's tissues. Due to a large number of OSCC biomarkers currently in use, non-invasive samples including blood, serum, and various other bodily fluids have a benefit over tissue samples [1].

Synthetic biology

Principles of synthetic biology are being investigated in the areas of genetic engineering, genome editing, and cancer immunotherapy. In order to modify the biological environment (by creating chimeric antigen receptors), and create synthetic oscillators, thereby changing the genome, synthetic biology has been expanded. It has been suggested that use synthetic networks as a cancer treatment technique. For instance, a microRNA-based cancer cell classifier makes use of microRNA expression to detect cancer cells and then initiate apoptosis.

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system for genome editing was formerly assumed to be the bacteria's developed resistance to viruses and plasmids. CRISPR is effective, simple to use, and commonly used in genome editing techniques [1].

CONCLUSION

The WHO has insisted on focusing majorly upon early diagnosis to reduce the number of deaths due to cancer, and there are a lot of developments since the last decade. Advanced, non-invasive and accurate devices or methods are the only hope to prevent cancer related deaths among the public. Early diagnosing gives a better prognosis, and it is important for a clinician to be up-to-date with such advancements for the betterment.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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