Early Detection of Precancerous and Cancerous Lesions: An Overview

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is the most common cancer of head and neck. A significant proportion of OSCC develop from premalignant lesions. The gold standard of assessing pathological changes in tissue is currently histopathology. In recent decades molecular method of disease diagnosis has gained importance as a rapid and simple method for obtaining DNA samples. Recent advances in the field of biologic science have sparked new interest in the areas of identifying cancer biomarkers in the bodily fluids. This paper reviews with advances in the techniques used for diagnosis of oral premalignant and malignant lesions.

Keywords: Cytology, Loss of heterozygosity, Microsatellite instability, Tumor markers, Spectroscopy.

INTRODUCTION

Oral cancer is the sixth most common malignancy worldwide. Over 300,000 new cases are reported annually. Two-third cases occur in the developing countries with only 5 years survival rate. India has the highest rate of oral cancer in the world. Over 92,000 cases of oral cancer are diagnosed every year. At the time of diagnosis most lesions are at stage 3. After every 7 minutes at least 1 death occurs in India due to oral cancer. WHO predicts continuing increase worldwide. With earlier detection, treatment is less complicated, the cosmetic and functional results are better and survival improved.

DIAGNOSTIC ADJUNCTS

Cytological Techniques

Dr George Papanicolaou is known as the father of cytology and the technique is called as PAP smear. Individual cells can often be diagnosed, as such microscopically by their large size, their pleomorphism, increased nucleocytoplasmic ratio, hyperchromatism and prominence of nuclei and their abnormal mitoses. Hundred percent accuracy is seen in lymph node aspiration from metastatic carcinoma, melanoma, Hodgkin’s and non-Hodgkin’s lymphoma. Oral cytology can give false-negative findings due to inadequate sampling.

Cyto Brush Technology

Leukoplakia, erythroplakia, chronic ulcerations, mucosa that is atrophic and common innocuous looking abnormalities can be checked for dysplastic cells. Whereas lesions with intact normal epithelium, fibromas, mucoceles, hemangiomas, submucosal masses, pigmented lesions and amalgam tattoos this technique cannot be used. For highly suspicious lesions, immediate scalpel biopsy is recommended.

Liquid Cytology

Transepithelial cells are harvested with a nylon bristle brush that is then immersed and twirled in a liquid preservative container. Better representation of collected lesional cells and easier interpretation is possible since there is a monolayer of cells with elimination of blood and debris.

Flow and Imaging Cytometry

Flow cytometric analysis has allowed detailed insights into the cellular biology of normal, reactive and neoplastic tissues. Lasers are the light source for clinical cytometry. The laser used is the argon type with 488 nm wavelength. This allows excitation of a variety of fluorochromes, phycoerythrin as well as certain DNA-bindind dyes. Here an instrument interrogates with individual cells with light so as to determine intrinsic (light scatter/cell size and granularity) and extrinsic characteristics (fluorescence/ antibody and nuclei acid dye binding) of the cells. Whereas in image cytometry, computerized image analysis is done for the conversion of continuous (digital) information, a form of data that can be stored and manipulated by digital computers.

Vital Tissue Staining

The use of toluidine blue (tolonium chloride) are used as an aid to the diagnosis of oral cancer and potentially malignant lesions as it will stain the abnormal (reactive and dysplastic) epithelium. Whereas lugol’s iodine will bind to glycogen present in normal epithelium, so is retained in...
normal squamous epithelial cells but not in dysplastic or malignant cells of the squamous epithelium.\(^3\)

**Tissue Fluorescence**

In fluorescence spectroscopy (FS) cells interact with light they become excited and re-emit light of varying color (fluorescence) which is detected by sensitive spectrometer. All tissues fluoresce due to the presence of fluorophores. Fluorophores are: NADH, collagen, elastin, cofactors such as flavins (FAD, FMN). Characteristic spectra reflect the biochemical change within the tissue. Dysplastic and malignant tissues shows increased red fluorescence and decreased green fluorescence. Whereas normal tissue has increased green fluorescence.\(^4\)

Raman spectroscopy (RS) refers to a form of inelastic scattering. It is based on the shift in the frequency of incident excitation light and the vibrational frequencies of biomolecules. Four principle components in biological tissue which is responsible for resultant spectra of excited light are: water, lipids (cell membrane), nucleic acids (DNA and RNA) and proteins (hormones, isoenzymes, immunoglobulins and keratins). This technique is extremely sensitive and most accurate. Limitations are that it is expensive, complex and difficult to adapt for in vivo use.\(^7\)

Elastic scattering spectroscopy (ESS), the scattered rays occur at different gradients or wavelength resulting due to the differences in densities that occur at cellular or subcellular level. Scattering centers are nucleus, chromatic concentration and subcellular organelles. ESS is sensitive to nuclear size, chromatic content, nuclear/cytoplasmic ratio and cellular crowding. It is fast, reliable, noninvasive, cost effective and diagnosis in situ can be easily done.\(^4\)

The product designed for this technique in the market are: VEL (visually enhanced lesion scope) scope and Microlux DL. The device is used in suspicious tissue using autofluorescence under diascopic pressure, i.e. applying a light amount of pressure in a sweeping motion with the back side of the explorer (or similar instrument) to diffuse any superficial blood from the area. If the green fluorescence returns with this pressure then the lesion may be of an inflammatory nature and inappropriate care to remove the possible cause may be recommended. The examination takes only 1 or 2 minutes and is a painless, noninvasive procedure. While camera adapter allows for photo documentation and tracking of the lesion. Limitations are that some benign conditions such as physiological pigmentation, amalgam tattoos, trauma, etc. may also appear as dark regions similar to malignancy.\(^4\)

**Chemiluminescence**

The product designed for this technique includes Vizilite plus or Vizilite with TBlue marking system. The patented single use vizilite test kit allows health care providers to improve early detection, evaluate and monitor oral mucosal abnormalities in those individuals at increased risk for oral cancer in a combination with a conventional oral mucosal examination. The disposable kit contains 54 mm plastic tube when flexed activates a nontoxic chemical light source. The activated vizilite inserted into its retractor, a 6-inch windowed hard plastic holder. Vizilite produces a diffuse light for approximately 10 minutes long enough to do a thorough visual oral examination. The kit also includes a small bottle of 1% acetic acid solution is used prior to the procedure to disrupt the glycoprotein barrier of mucosal surfaces. Vizilite passes over oral tissue that has been treated with rinse solution. Normal healthy tissue will absorb the light and appear dark, abnormal tissue will appear white.\(^8\)

**MICROENDOSCOPY**

Andreas first used endoscope for use in the upper aerodigestive tract. The microendoscope has fitted rotating screw which allows magnification to be changed from 0× to 150×. Gives enough magnification of up to 300 times to 1,000 times. The methodology for microendoscopy is same as direct oral microscopy. 0.1% methylene blue is used as vital staining agent. The tip of microscope is applied to the pre-stained area of interest for assessment of underlying mucosal vasculature and blood flow.\(^3\)

**Direct Oral Microscopy**

Helps in selecting more representative site for biopsy. It uses a specialized mobile microscope with magnification of 0×, 12× and 20×. Then lens with a green filter is used to examine the vascular changes and color tissue. 1% acetic acid is applied to the lesion for 5 seconds and dried with low-pressure airflow.\(^4\)

**DIAGNOSTIC METHODS**

**Biopsy**

The goal of excision biopsy is to obtain the entire abnormality for histopathological examination and to provide definitive treatment by the total removal of the lesion. It is reserved for clinically benign and precancerous mucosal lesions that are less than 2 cm in diameter. For lesions greater than 3 cm in diameter, the use of multiple incisional biopsies and vital staining may be warranted to help indentify or exclude focal carcinomatous transformation. Punch biopsy can be considered for deep biopsies in the areas like palate can be relatively simple to obtain.\(^4\)
Imaging

Imaging options include panoramic radiography, CT, MRI and positron emission tomography. Plain film radiograph may only demonstrate gross bony involvement. Computed tomography with intravenous contrast are the most common imaging modality used in the assessment of deep tissue extension of tumors of oral cavity. It clearly demonstrate bone changes and tumor invasion and is more sensitive than MRI for lymphadenopathy. Magnetic resonance imaging is superior in providing soft tissue details. Has multiplanar imaging capability which can better demonstrate intracranial extension of the tumor. Limitations are that bony details are not clear and cannot to be used in patients who cannot easily lie still or are claustrophobic. Ultrasound helps in evaluation of neck nodes along with the size and is a useful guide in performing FNAC for obtaining cytology. However, bone involvement cannot be evaluated as bone does not transmit sound.1

Positron emission tomography (PET) is a form of nuclear medicine study and is used to identify nodal metastasis and recurrent tumor. PET is based on positron generation from a positron heavy nucleus. Fluorodeoxy-glucose is an imaging isotope used in PET using the difference in metabolism of radiolabeled glucose molecules between normal and malignant tissues. PET is proving to be even more specific and sensitive than CT and MRI in detection of recurrent tumors and distinguishing tumors from post radiation therapy effects.3

Sentinel Node Biopsy (SNB)

This procedure is intended to identify micrometastastic disease within a ‘sentinel’ node considered most likely to drain the tumor bed and receive initial metastatic deposits from the primary malignancy. Represents a less invasive means of providing staging information for the patients with oral cancer with an N0 neck.1

Molecular Methods

Recent advances in molecular studies show that an alteration at DNA level precedes microscopic morphologic changes in precancer and cancer. Evaluation of these markers may help in early characterization of oral epithelial dysplasia and squamous cell carcinoma.

NUCLEOLAR ORGANIZING REGIONS

These are loops of ribosomal DNA located on the short arms of chromosome 13, 14, 15, 21 and 22 and are associated with acidic nonhistonic proteins that can be visualized by silver staining techniques. Number and size of AgNORs correlates positively with cellular proliferation. Mean AgNOR counts differ significantly between nondysplastic and dysplastic clinical leukoplasia. With a sensitivity of 75% and specificity of 83% with a cut off mean AgNOR value of 2.37. Limitation with the technique include the time and effort required to perform the study manually as well as staining variability and counting subjectivity.6

Abnormal DNA Segregation (DNA aneuploidy) and Gene Alterations

Abnormal chromosomal segregation resulting in aneuploidy can be marker for neoplastic transformation. Most of the oral cavity carcinogens are chemical (tobacco), physical (radiation), and infectious (human papilloma virus, candida) mutagenic agents that may cause changes in gene and chromosome structure by point mutations, deletion, insertions and rearrangements. Mutations in the p53 are the most frequent genetic alterations in human cancer and show a variable frequency in oral cancer.9

Epigenetic Alteration, Loss of Heterozygosity (LOH) and Microsatellite Instability

The applicability of other molecular markers, such as epigenetic alterations (hypermethylation of promoter regions) and genomic instability, such as loss of heterozygosity and microsatellite instability (MSI). Rosas et al studied the methylation patterns of p16, MGMT and DAP-K genes in smears of patients suffering from head and neck cancer. They detected abnormal hypermethylation patterns in both kinds of samples by a methylation specific polymerase chain reaction (PCR).10 Huang et al used PCR techniques to amplify DNA from exfoliated cytology samples from oral carcinomas for analysis of restriction-fragment length polymorphisms (RFLPs). They found that 66% of the tumors studied showed LOH at one position in the p53 sequence, while 55% showed LOH at some other location. Microsatellite regions are distributed along the genome and have been widely and satisfactorily used as molecular markers for carcinogenesis.11

Viral Genome Studies

RNA oncogenic viruses transform cells by two mechanisms. Acute transforming viruses containing a transforming viral oncogene (v-onc), such as V-SRC, V-ABL and slow transforming viruses (e.g. mouse mammary tumor virus) which do not contain a v-onc, but the proviral DNA is
always found inserted near a cellular oncogene. Under the influence of a strong retroviral promoter, the adjacent normal or mutated cellular oncogene is overexpressed. This mechanism of transformation is called insertional mutagenesis. Human T-cell leukemia virus-I (HTLV-1) is associated with a form of T-cell leukemia/lymphoma similar to the acquired immunodeficiency syndrome (AIDS) virus, HTLV-I has tropism for CD4+ T cells, and this subset of T cells is the major target for neoplastic transformation.10

DNA oncogenic viruses, such as human papilloma virus (HPV), Epstein-Barr virus (EBV), human herpes virus 8 (HHV-8, Kaposi sarcoma) and HBV are strongly associated with human cancer. HPV's have been implicated in the genesis of several cancers, particularly squamous cell carcinoma of the cervix and anal, perianal, vulvar, and penile cancer. Emerging evidence indicates that about 20% of oropharyngeal cancers are HPV-associated. DNA sequences of HPV types 16 and 18 are found in 75 to 100% of invasive squamous cell cancers (i.e. severe dysplasias and carcinoma in situ). EBV has been implicated in the pathogenesis of several human tumors: Burkitt lymphoma, post-transplant lymphoproliferative disease, primary central nervous system lymphoma in AIDS patients, a subset of other AIDS-related lymphomas, a subset of Hodgkin lymphoma, and nasopharyngeal carcinoma. More than 90% of African tumors carry the EBV genome. One hundred percent of the patients have elevated antibody titers against viral capsid antigens. Serum antibody titers against viral capsid antigens are correlated with the risk of developing the tumor. Burkitt lymphoma have a t(8;14) or, less commonly, variant translocations that lead to dysregulated expression of the c-MYC oncogene.9

Immunohistochemical Identification of Tumor Markers

The identification of tumor markers, notably cytokeratins in smears from the oral cavity has attracted considerable interest. Cytokeratin expression profile provides useful information on cell differentiation status but its potential for early diagnosis of oral cancer is limited. However, certain cytokeratins, such as K8 and K19 are useful if not definitive indications for malignancy, particularly if their presence is interpreted in conjunction with other information such as DNA profile.12

The Future

It has also been shown that tumor markers can be detected in the serum and more recently the saliva of patients with cancer. Seven salivary mRNA’s are proposed as candidate cancer related biomarkers including interleukin (IL)-8, IL-1B, DUSP 1, HA 3, OAZ 1, SIDOP and SAT. Serum analysis by mass spectrometry was recently used to evaluate oral squamous cell carcinoma patients.13

CONCLUSION

Technology is poised to play an active role in the diagnosis of patients with precancerous and cancerous lesions. The advanced techniques are right now used at a handful of research centers. Until more laboratories acquire needed molecular technologies, routine histopathologic examination is likely to remain the standard for detection of most patients with oral cancer.

REFERENCES

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