ABSTRACT

The pathology has extended its wings in the past few decades and has contributed greatly in understanding the pathogenesis of genetic disorders and in diagnosis of several undifferentiated malignant neoplasms. Molecular techniques are being used in clinical field. There are various techniques which are introduced in the field of pathology like brush cytology, veselcope, confocal microscopy, tumor markers, microarray etc. New emerging technologies, including robotics, humanoid technology, lab-on-chip devices, nanodevices and patient ‘smart’ implants, will in the future offer unique opportunities for laboratories to develop. In present article few of the various techniques have been discussed which when used optimally by the pathologist have improved the quality of life of patients and newer future diagnostic techniques which could be boon in the field of pathology.

INTRODUCTION

The first microscopic description of human tumors were found in a text by Sir Edward Home in 1830, followed by Johannes Muller in 1838. The term “biopsy” was coined by Ernest Besnier in 1879. There was restricted application of biopsy until the mid-20th century. Wax embedding of specimens did not come into general use till the 1800s and histotechnology was still not in function. Frozen sections were being introduced in American medical centres in twentieth century. According to Pearse a French botanist Francois-Vincent Raspail was the first to demonstrate the chemical reaction with the microscopic observation of tissues and cells. (Medhini Singaraju et al., 2013; Underwood, 1987) According to Pearse a French botanist Francois-Vincent Raspail was the first to demonstrate the chemical reaction with the microscopic observation of tissues and cells. (Medhini Singaraju et al., 2013; Underwood, 1987)

Vital Tissue Staining – Toluidine Blue Staining & Lugol’s Iodine

Early malignant lesions show affinity for toluidine blue dye. Lugol’s iodine and toluidine blue have been used together in the detection of early carcinomas and other oral lesions. Toluidine blue is an (acidophilic) metachromatic dye which selectively stains acidic tissue components, thus staining DNA and RNA. It stains mitochondrial DNA, cells with greater than normal DNA content or altered DNA seen in dysplastic and malignant cells. Lugol’s solution is used for delineation of the malignant change which produces a brown black stain when the iodine reacts with the glycogen content. The use of toluidine blue and Lugol’s iodine serves as a useful adjunct in the diagnosis of patients who are at risk and for selecting the site for biopsy with wide field cancers prior to treatment. (Underwood, 1987; Masthan et al., 2012) Toluidine blue has also been reported as an aid in selecting biopsy sites and in delineating the margins of lesions (Chaudhary et al., 2014).

Chemoluminescence – Vizilite

This device has been approved for use in the United States by the Food and Drug Administration (November 2001). It has been investigated that the use of vizilite is beneficial as compared to Conventional visual examination. This is a handheld, single-use, disposable chemiluminescent light stick that emits light at 430, 540 and 580 nm wavelengths. Normal epithelium will absorb light and appear dark whereas hyper keratinized or dysplastic lesions appear white. (Chaudhary et al., 2014; Joel et al., 2002; Diana V Messadi, 2013) It is recommended to use dilute acetic acid rinse and observe under a chemiluminescent light such as vizilite for improved early
detection of oral cancer (Masthan et al., 2012). A recent study of high risk patients showed that the majority of lesions with a histological diagnosis of dysplasia or carcinoma in situ were detected and mapped using vizilite with TB (Joel et al., 2002).

**Brush cytology (Oral CDX)**

Oral cdx is based on the concept of exfoliative cytology in the assessment of dysplastic changes in various suspected lesions especially in oral cancer. The use of brush cytology is inexpensive, simple, noninvasive and also risk-free technique. The oral epithelial cells can be obtained by the use of a cytobrush (thenon lacerational device), samples are fixed onto a glass slide, stained with a modified Papanicolaou test and analyzed microscopically via a computer-based imaging system. With brush cytology, sensitivity for detecting oral epithelial dysplasia but is associated with controversies and false negative results. (Underwood, 1987; Chaudhary et al., 2014; Masthan et al., 2012)

**Velscope (narrow emission tissue fluoroscence)**

It has been used in screening and diagnosis of precancer and early cancer of lung, uterine cervix skin and oral cavity. (Diana V Messadi, 2013) The concept of tissue autofluorescence in diagnosis of dysplastic lesions of the oral cavity is based on changes in the structure and metabolism of the epithelium and the subepithelial stroma when interacting with light. Specifically, loss of autofluorescence in dysplastic and cancerous tissue is believed to reflect a complex mixture of alterations to intrinsic tissue fluorophore distribution, due to tissue remodeling such as the breakdown of the collagen matrix and elastin composition. Normal mucosa emits pale green autofluorescence. (Underwood, 1987; Diana V Messadi, 2013; Masthan, 2012). It act as a adjunct in improving distinction benign and malignant changes and identifying the malignant lesion that are not visible to naked eye under white light. (Diana V Messadi, 2013)

**Confocal microscopy**

This is an imaging technique for various researches in cell biology with an advantage of optical sectioning and high resolution imaging. This technique is useful in identifying the characteristic features such as nuclear irregularity which is used to differentiate OSCC from normal oral mucosa. However, further optimization of the instrument is still required. (Masthan, 2012)

**Optical Coherence Tomography (OCT)**

It was first reported by Fujimoto et al. (1991), it has numerous applications gastroenterology, ophthalmology, dermatology, and dentistry. OCT is a non-invasive, non-radiative optical diagnostic tool based on interferometers. (Underwood, 1987; Gaikwad et al., 2013) There are few indicators involved in diagnosis of oral cancer, like the EP layer thickness and the standard deviation (SD) of OCT signal intensity. In an abnormal oral EP containing dysplastic cells, the cell size, shape, nucleus size, and arrangement is more randomly distributed as compared to healthy oral epithelium (EP). In this scenario, light scattering becomes stronger and its spatial distribution becomes more strongly fluctuated (Gaikwad et al., 2013) The advantage of OCT is cross-sectional images of normal or abnormal tissues can be obtained without biopsy and there is no exposure of the patient to ionizing radiation.

**Tumor Markers & Bio Markers**

Tumor markers may be present in blood circulation, body cavity fluids, cell membranes and cell cytoplasm. When released by cancer cells or produced by the host in response to cancerous substances. (Masthan et al., 2012) The most reliable biomarker in OSCC development include the TSG p53 protein expression, chromosomal polysomy (DNA ploidy), and changes (termed loss of heterozygozity; LOH) in chromosomes 3p or 9p (probably due to changes in the TSG p16). Tumour Suppressor Genes, Oncogenes, cell proliferation markers, angiogenic markers and cell adhesion molecules are useful in prediction for the prognosis of patients with OSCC. (Masthan et al., 2012) Molecular analysis offexfoliated cells also has shown the same changes as are present in tumor biopsy specimens. (Chaudhary et al., 2014)

**PCR-Based diagnostic aids**

It can be used to detectmutations in cancer-associated oncogenes (e.g., K-ras, Nras), tumor suppressor genes (e.g., p53, p16) etc. and aids as an important detection tool. With PCR technique the range and sensitivity ofdiagnostic procedure has been increased. But still has a major drawback, of contamination and amplification artefacts may give rise to difficulties in the interpretation of the desired results. (Masthan et al., 2012)

**IDENTAFI 3000**

This technology is a combination of anatomical imaging with fluorescence, fiber optics and confocal microscopy to map and delineate precisely the lesion in the area being screened. It is small in size and easy accessibility to all tissues in the oral cavity. The mechanism is similar to veloscope and also detect changes in angiogenesis with green amber light illumination. (Joel B. Epstein et al., 2002)

**Microarray**

The pattern of gene expression vary in normal tissue and its malignant counterpart, which can be assessed by Tissue microarray technique. (Nigam et al., 2014) RNA from malignant lesion and from a control tissue are extracted, and cDNA's are prepared by reverse transcription. These reverse transcription reactions incorporate different fluorescent dyes so that cDNA from the tumor and from the control tissue can be distinguished by their fluorescence emission spectra. These cDNAs are mixed and then hybridized to the microarray. A relative increase in expression of a particular gene in a tumor sample leads to an increase in binding of labeled tumorderived cDNA to the spot in the array that is complementary to the gene of interest. DNA microarrays are being used to detect single nucleotide polymorphisms (SNPs) of our genome (Hap Map Project), aberrations in methylation patterns, alterations in gene copy number, alternative RNA splicing, and pathogen detection. (Nigam et al., 2014; Trevino et al., 2007)

**Future of diagnostic techniques**

LAB ON CHIP- lab-on-a-chip or micro-total-analysis systems (TAS) also known as Microfluid technology. It is the
Dried blood spot

The test uses a dried blood spot specimen. It works by converting protein to peptides and then using a mass spectrometer to select and accurately measure diagnostic metabolites and/or peptides. It requires only a tiny blood sample. Genetic testing and molecular diagnostic assay can be performed from DBS specimen. 

Virtual colonoscopy

It uses CT technology as an alternative to optical screening colonoscopy. VC digitally reconstructs the CT image into 2D and 3D pictures of colonic luminal surfaces.

Conclusion

In the future hopefully, pathologist will not be merely “tissue sampler”. Research will be focused on cancer prognostic markers and cancer cures via targeted therapies. Advances in molecular taxonomy will be an important adjunct to histologic diagnoses. Development of tissue banks, construction of microarrays, molecular research will be the framework in future of research pathology. Advances in genetics, information technology and digital imaging are already transforming histopathology and many other pathology specialties. Much interpretive reporting will be done from flat screens rather than through microscope eyepieces. Molecular diagnostic, genomics and proteomics will have the high impact because they will continue to redefine disease at the molecular level. The further development of nanotechnologies will also drive change and expedite diagnoses. The other changes will be increased automation; more things can be done with greater consistency, less time and less cost. In the developed world, pathology will still use many of the current techniques but these will be enhanced due to development predominantly in molecular pathology, information technology and imaging techniques and by commercialisation of new techniques and better instrumentation.

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