ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 04 | April - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANALYSIS OF VITAL STAINING: A NARRATIVE REVIEW

Oral Pathology

Dr. R. Arun Amarnath CRI, Adhiparasakthi dental college and hospital, Melmaruvathur.
Dr. Adhithya Baskaran Assistant Professor, Adhiparasakthi dental college and hospital, Melmaruvathur.
Dr. Kokila Sivakumar Assistant Professor, Adhiparasakthi dental college and hospital, Melmaruvathur.

ABSTRACT

Vital staining is a method used in biology and medicine to specifically stain living cells or tissues in order to examine their makeup, activity, and functions. The stains used in vital staining can be used to emphasise characteristics like nuclei, mitochondria, or particular proteins. These stains are frequently specific to particular structures or substances within cells. There are many uses for vital staining, ranging from fundamental studies of cell biology and disease processes to clinical diagnosis and monitoring of medical conditions. In order to satisfy the demands of various research questions and clinical applications, novel staining agents and methodologies have been developed over time. Vital staining is still an effective method for analysing living cells and tissues, despite its drawbacks and possible hazards. Even though there are numerous vital stains discovered Toluidine Blue is more commonly used in practice .

KEYWORDS

Specifically Stains Living Cells, Intravital Staining, Supravital Staining .

INTRODUCTION

Vital stains are stains that can be administered in living cells which does not kill the living cells(1). In the field of oral pathology vital staining is used as important diagnostic tool(1). This technique is employed to improve early detection and diagnosis for oral malignancies(2). Cell components that be stained by vital staining are mitochondria , lipid vesicles, lysosome etc(3). Commonly vital staining is done for patients who are in state of developing oral cancer and with confirmed state of neoplasm (4). Though oral malignancies are life threatening condition it is often treatable when it is diagnosed at early stage (5). Oral Lesion and condition that can be vitally stained are erythropakia, leukoplakia , discord lupus erythematosus, palatal lesion of reverse cigar smoking, OSMF, epidermolysis bullosa, and dyskeratosis congenita (6). Application of vital stains in mucosa of Tobacco users shows dysplastic changes on histological examination (7).

Classification:

Basically vital staining is classified into two types
1. Intravital staining { in vivo}
2. Supravital staining{in vitro }(3)

Principle:

The idea behind vital staining is that macrophages, or phagocytic cells, consume colored matter particles. Phagocytosis may be the source of the dye's uptake. Additionally, the colored particle can be seen in the cell's cytoplasm.(1)

Types

1.Intravital Staining

- Toluidine Blue
- methylene blue
- Rose Bengal
- Lugols iodine
- Acetic acid

2. Supravital Staining

- Janus green stain
- DIOC for endoplasmic reticulum
- Nile red
- Rhodamine 123
- Tryphan blue(1)

1. Intravital Staining

TOLUIDINE BLUE(TB)

Mechanism:

TB is known as tolonium chloride. Its chemical formula is $C_{15}H_{16}N_3S^+$. It can dissolve in alcohol up to 0.5 percent and up to 3.5% in water. TB is an acidophilic dye which belongs to thiazine group that specifically colors acidic tissue components, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), as well as carboxylates, sulfates, and phosphate radicals. Due to the repeated phosphate groups in the nucleic acids, it has the staining characteristic known as metachromasia, which is influenced by temperature and pH. The pH range is 6.0 to 7.0. The metachromatic characteristic weakens after 30°C, so the temperature shouldn't go above that.

A cationic dye, TB binds to the DNA's nucleohistones in two different methods. Intercalation is one technique, and aggregation or stacking is another. The amount of DNA present is correlated with the quantity and size of nuclei found in the superficial layers, and the amount of DNA binds to phosphate bonds. Its application in living organisms is justified by the quantitatively higher nucleic acid content of dysplastic and anaplastic cells compared to normal tissues, as well as the loss of cell cohesion and greater mitosis.

Additionally, malignant epithelium may have larger intracellular canals, which improves the dye's ability to penetrate the tissue. It only represents changes in the epithelium and cannot be used to detect invasion into the connective tissue beneath or changes in the submucosa because it only stains to a depth of two to ten cell layers.(8)

Composition:

- 100 ml of 1% TB contains
- toluidine blue Powder -1gm
- 1% acetic acid – 10ml
- absolute Alcohol -4.19ml
- distilled water 86ml and pH at 4.5.(3)

Technique:

To remove the debris, the patient is initially instructed to rinse his lips twice with water for 20 seconds each time. After that, 20 seconds of 1% acetic acid are administered to wash away any ropey spit. When a mucosal lesion is visible 1% TB solution is then applied for 20 seconds with a cotton brush or administered as a rinse. To lessen the severity of mechanically held stain once again 1% acetic acid was administered. Finally, water is used to rinse the mouth.(5)

Interpretation:

Positive staining it exhibits a dark blue (royal or azure) stain on all or part of the lesion; negative staining is the absence of color absorption by the lesion; and doubtful staining is the presence of a light or pale blue stain.(8)

METHYLENE BLUE (MB)

Mechanism:

The precise mechanism underlying methylene blue uptake in epithelial tissue may mask toluidine blue uptake in acidophilic characteristics of cells with nucleic acid concentration is abnormal and results in dissimilar absorption between dysplastic cells and normal cells. It was at least 72-100% responsive for MB's 90% sensitivity.(6)

Compostion:
- There were two solution vessels in the Methylene Blue dye system.
- The color rinse solution (Bottle A) contained
- Glycerol
- Dimethylsulfoxide,
- 0.5% eosin,
- 1% methylene blue,
- 1% malachite
- Bottle B contained filtered water and 1% lactic acid for the pre- and post-rinse solutions.(6)

Technique:
Methylene blue was used in the following ways. The patients were instructed to rinse their mouths for 30 seconds with 1% lactic acid and distilled water to get rid of food particles and extra saliva as well as to maintain a constant oral environment. To make sure that the lesion was not being contaminated with saliva, the mucosa of the target region was carefully dried with gauze and air spray. Using a cotton swab, the dye was first applied directly to the lesion. Next, Methylene blue was gargled for 30 seconds in the mouth before being expectorated. To remove the extra dye, patients then rinsed with 1% lactic acid again for 30 seconds.(6)

Interpretation:
The degree of stain retention on the lesion was used to gauge the pattern of color retention. Positive (+) reactions were indicated by local, stippled, patchy, and deep blue spots. Negative (-) reactions were indicated by wide, shallow, or light blue spots. Bottle B solution was used to remove the staining surface for ambiguous staining after being applied with cotton rolls. A unfavorable response was noted if the blue stain was removed, and the opposite was true.(9)

LUGOLS IODINE (LI)
Mechanism:
In intermediate-level cells the cytoplasm of the outermost cells contains glycogen.Conversely, aggressive cancer cells and dysplastic tumors contain only there is no glucose. Due to iodine's affinity for glycoproteins regular cells uptake more iodine when Lugol's iodine solution is administered. Due to lack of glucose, cancer cells cannot absorb iodine and manifest golden like mustard or saffron colour .(10)

Composition:
- Potassium iodide – 4 Grams
- Distilled water – 100 cc
- Iodine – 2 grams(1)

Technique:
Cleaning the wound with a cotton tip dipped in 10% H_2O_2 (to remove any remaining saliva, food, or tissue),Using a water jet clean the lesion, 30 seconds of cotton-tipped application of Lugol's iodine solution.(10)

Interpretation:
Lesions with a brown stain were regarded as positive, when the lesion is unstained it is considered as negative. The biopsy location was chosen based on dye retention and it's appearance. In cases where there was no stain retention in the lesion biopsy was guided.(11)

ACETIC ACID
Mechanism:
As a consequence of osmolar changes caused by acetic acid, water moves out of the cells. Consequently, this causes dehydration, which ultimately causes the cell to disintegrate. Barrier surrounding an enlarged, abnormal nucleus, and cellular protein coagulation. This makes the epithelium less transparent, making the lesion look white. (7)

Composition:
100 ml of 1% acetic acid rinse contains 1 ml of glacial acetic acid with 99 ml distilled water.(3)

Technique:
Everytime newly prepared 5% acetic acid was used. An already cleansed and dried mucosa or lesion was treated with acetic acid-soaked gauze. Subjects were instructed to rinse their mouths with the same solution for bigger and more widespread lesions. The lesion has been scanned again after two minutes.(7)

Interpretation:
Due to light reflection from the underlying, blood vessel-rich stroma , columnar epithelium looks red and the normal squamous epithelium looks pink , giving them their distinctive colors. Coagulation of cellular proteins occurs when it contacts with acetic acid , which alter the stroma's color. When contrasted to the surrounding normal squamous epithelium's pinkish color, the resulting aceto-whitening is clearly visible.(1)

ROSE BENGAL stain (RB stain)
Mechanism:
Rose Bengal (RB), a 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodo-fluorescein derivative, is frequently used to identify a variety of ocular surface conditions. It is thought that the staining occurs more often in areas where the preocular tear film fails to adequately protect the surface epithelium, such as desquamated ocular epithelial cells, dead or degenerated cells, but not healthy epithelial cells, or in areas where there is a loss of cell vitality. Even the breadth of corneal and conjunctival tumors could be determined using RB staining.(12)

Technique:
(i) rinse the mouth with distilled water (reagent A) for one minute to clean the lesions , (ii) using cotton tip apply the RB solution (reagent B) for 2 minute, (iii) to remove excess RB solution rinse the mouth with distilled water for 1 Minutes, (12)

Interpretation
Pink: positive; no hue change: negative .False negative outcomes may arise from the cells' late clinical expression of genetically induced changes, and flourescent properties are comparable to DAPI. Living and unfixed cells are stained by RB .The wavelengths with the highest levels of emission and absorption produce blue light at 450 nm and 340 nm, respectively.(1)

2. Supravital Staining
DiOC3:
DiOC3 is a green fluorescent dye that is lipophilic, cationic, and used to label ER in a broad range of cells. This planar dicarbocyanine pigment molecule demonstrates hydrophobic and hydrophilic properties. According to tradition, the letters “di” and “T” stand for the identical halves of the molecule, “3” stands for the three carbon chains connecting the cyclic halves of the molecule, and “0” stands for the two oxygen atoms in the rings.(13)

Activity in Cancer
Organic dye photosensitizers that selectively kill cells are a significant field of research with expanding clinical applications to cancer therapy. Using photodynamic therapy as a novel approach to treating cancer is beneficial. It has been investigated how photosensitization with DiOC3 affects cellular biology. DiOC3 photosensitization selectively damages cell microtubules and results in a highly localized, seemingly complete inhibition of ER motility.(13)

Janus Green Stain:
A supravital monoazo dye known as Janus green B has been demonstrated to be easily adaptable to cytotoxicity research due to its capacity to accumulate only in dead cells. The Janus green technique has only ever been used to evaluate the viability of corneas. To make the Janus Green method more reliable and useful for cell culture applications, we significantly modified it.When cell layers treated with Janus green are inspected under a microscope, the plasma only shows weakly stained structures, while the cell nuclei look deeply blue stained. Actually, cationic dyes must be sensitive to changes in their contents because they are electrostatically attach to negatively charged macromolecules groups like DNA, or RNA glycosaminoglycans. Hence Janus green exhibits different staining characteristics during the lag period of cells, which is a stage of total cellular reorganization.(14)

RHODAMINE 123(Rh-123):
Eastman Organic Chemicals provided the Rh-123. For the creation of all solutions and standards, a 10 mg/ml stock solution in dimethyl sulfoxide was stored in the dark at 4 °C. (15)In numerous

investigations of mitochondria in living cells, the permeant cationic fluorochrome Rh-123 has been used. Rh-123 gives off radiation at non-toxic levels, which Mitochondria in various cell types can be seen in low background, high density fluorescent images. Preferred building and it appears that the mitochondrial membrane potential and the preservation of this dye by mitochondria are correlated. Studies of cells going through changes in their metabolic condition have used Rh-123 as a marker of mitochondrial membrane potential. Rh-123 may also show a distinction in quality between the mitochondria of healthy and altered cells. After being transferred to dye-free medium, mitochondria of feline sarcoma virus-transformed mink fibroblasts maintain noticeably less Rh-123 than do mitochondria of the parent cells that did not undergo transfection.(16)

Tryphan Blue:

Trypan blue is a crucial stain After staining when it is viewed under a microscope, nonviable cells appears blue , whereas viable cells look unstained. This is due to the viable cells have undamaged cell membranes, so they cannot absorb dye from the surrounding medium. However, non-viable cells do absorb dye from their environment because they lack a functional and intact membrane.Trypan blue solution: 0.4% prepared in 0.81% sodium chloride and 0.06% dibasic potassium phosphate.As a result, it is simple to differentiate between non-viable and viable cells because the former are small, round, and unstained, whereas the latter are stained and swollen. Apoptotic and necrotic cells are not distinguished by the technique.(17)

NILE RED:

Nile blue was first used by Smith in 1907 to histochemically identify cellular lipids. He discovered that nile blue and related phenoxazine dyes had the remarkable characteristic of staining acid lipids blue and neutral lipids red at the same time. The same year, Thorpe looked at the chemical makeup of nile blue and other blue phenoxazine dyes and discovered that each one of them contained different amounts of oxidation byproducts known as phenoxazones.Nile red demonstrates characteristics of a lysochrome that are very close to ideal. Only in the presence of a hydrophobic environment does it exhibit intense fluorescent properties. The pigment interacts with no tissue component other than through solution and is highly soluble in the lipids it is meant to display. Nile Red can be used to stain cells in an aqueous medium, and it does not dissolve the lipids that it is meant to expose. (18)

Under fluorescent microscopy, it gives the cell's lipid vesicles a red color and stains them. (1)

CONCLUSION

Vital staining is cost effective procedure that cause minimal discomfort to the individual and also help to locate the appropriate site for biopsy, Early detection of pathology can help to decrease the death rate, it is a simple procedure which does not need skillful operator, more studies are needed in intravital and supravital staining of various tissue samples to unserstand the nature about the stain.

REFERENCES

1. Nitya K, Amberkar VS, Nadar BG. Vital Staining-Pivotal Role in the Field of Pathology. Ann. Cytol. Pathol. 2020;5:058-63.
2. Patton LL, Epstein JB, Kerr AR. Adjunctive techniques for oral cancer examination and lesion diagnosis: a systematic review of the literature. The Journal of the American Dental Association. 2008 Jul 1;139(7):896-905.
3. Bagalad BS, Kumar M. Vital staining: Clinical tool In discovering oral epithelial dysplasia and carcinoma-overview. J Dent Pract Res. 2013;1(1):34-8.
4. Sridharan G, Shankar AA. Toluidine blue: A review of its chemistry and clinical utility. Journal of oral and maxillofacial pathology: JOMFP. 2012 May;16(2):251.
5. Hedge MC, Kamath PM, Shreedharan S, Dannana NK, Raju RM. Supravital staining: It's role in detecting early malignancies. Indian Journal of Otolaryngology and Head & Neck Surgery. 2006 Jan;58(1):31.
6. Riaz A, Shreedhar B, Kamboj M, Natarajan S. Methylene blue as an early diagnostic marker for oral precancer and cancer. Springerplus. 2013 Dec;2(1):1-7.
7. Vinuth DP, Agarwal P, Kale AD, Hallikeramath S, Shukla D. Acetic acid as an adjunct vital stain in diagnosis of tobacco-associated oral lesions: A pilot study. Journal of Oral and Maxillofacial Pathology: JOMFP. 2015 May;19(2):134.
8. Sudheendra US, Sreeshyla HS, Shashidara R. Vital tissue staining in the diagnosis of oral precancer and cancer: Stains, technique, utility, and reliability. Clin Cancer Investig J. 2014 Mar 1;3:141-5.
9. Chen YW, Lin JS, Wu CH, Lui MT, Kao SY, Fong Y. Application of in vivo stain of methylene blue as a diagnostic aid in the early detection and screening of oral squamous cell carcinoma and precancer lesions. Journal of the Chinese Medical Association. 2007 Nov 1;70(11):497-503.
10. Fatima S, Basu R, Hallur NH. Lugol's iodine identifies dysplastic tissue in precancerous lesions: A clinical trial. Annals of Maxillofacial Surgery. 2016 Jul;6(2):172.
11. Kamarthi N. Diagnostic efficiancy of toluidine blue with Lugol's Iodine in oral premalignant and malignant lesions (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
12. Du GF, Li CZ, Chen HZ, Chen XM, Xiao Q, Cao ZG, Shang SH, Cai X. Rose bengal staining in detection of oral precancerous and malignant lesions with colorimetric evaluation: a pilot study. International journal of cancer. 2007 May 1;120(9):1958-63.
13. Sabnis RW, Deligeorgiev TG, Jachak MN, Dalvi TS. DiOC6 (3): a useful dye for staining the endoplasmic reticulum. Biotechnic & histochemistry. 1997 Jan 1;72(5):253-8.
14. Raspotnig G, Fauler G, Jantscher A, Windischhofer W, Schachl K, Leis HJ. Colorimetric determination of cell numbers by Janus green staining. Analytical biochemistry. 1999 Nov 1;275(1):74-83.
15. Modica-Napolitano JS, Aprille JR. Basis for the selective cytotoxicity of rhodamine 123. Cancer research. 1987 Aug 15;47(16):4361-5.
16. Nadakavukaren KK, Nadakavukaren JJ, Chen LB. Increased rhodamine 123 uptake by carcinoma cells. Cancer research. 1985 Dec;45(12_Part_1):6093-9.
17. Louis KS, Siegel AC. Cell viability analysis using trypan blue: manual and automated methods. Mammalian cell viability: methods and protocols. 2011:7-12.
18. Greenspan P, Mayer EP, Fowler SD. Nile red: a selective fluorescent stain for intracellular lipid droplets. The Journal of cell biology. 1985 Mar;100(3):965-73.