

Toluidine Blue Staining in The Diagnosis of Oral Precancer and Cancer :Stains, Technique and its Uses - A Review

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DOI: <http://dx.doi.org/10.13005/bpj/737>

(Received: August 15, 2015; accepted: September 20, 2015)

ABSTRACT

Toluidine blue is a basic thiazine metachromatic dye with high affinity for acidic tissue components, thereby staining tissues rich in DNA and RNA. Toluidine blue has been used *in vivo* to identify dysplasia and carcinoma of the oral cavity. This article reviews the various vital tissue staining techniques available in the diagnosis of oral precancer and cancer.

Key words: Diagnosis, oral cancer, toluidine blue vital stain.

INTRODUCTION

Cancer may be defined as an uncontrolled tissue growth in susceptible patients, which results from an imbalance between cell division and programmed cell death (apoptosis). The factors implicated as potential “initiators” and/or “promoters” of cancer are: tobacco, alcohol, solar radiation, ionizing radiation, occupational carcinogens, environmental pollutants, medications, infectious agents, and nutrients¹. The prevalence of oral cancer in India is up to 4 times higher than in other countries.² Estimates indicate 57% of all men and 11% of women between 15–49 years of age use some form of tobacco. An increasing ages; however, lower incidence recorded amongst females as compared to males is indicative of gender differences in the lifestyle and behavioural patterns associated with incidence of oral cancer.³ In the USA, cancer incidence and associated mortality have declined due to improved health education and awareness translating to improved prevention, earlier detection and availability of treatment options⁴ Toluidine blue (TB)

application is believed to be a reliable indicator for identifying early malignant transformation. This is much cost effective with fewer false negatives and has a potential as a mass screening tool. Though several studies have documented the efficacy of TB in cervical lesions, only few studies are available to predict the use of TB for predicting oral potentially malignant states (OMPS) grades. Apart from TB, other stains such as methylene blue (MB), Lugol's iodine, and acetic acid have also been tried in the diagnosis of cancerous lesions.

TB

TB was first used by Richart in 1963 to stain uterine cervical carcinoma *in situ* and dysplasia.⁵ There are various important research which are needed for the diagnosis and treatment plan of various disorders related to oral cavity. The use of toluidine Blue (toluidine chloride) dye as a mouthwash is currently receiving much attention as an aid to the diagnosis of oral cancer and potentially malignant lesions. The method has good sensitivity with a very low false negative rate.⁶

This study is mainly designed to know the following conditions

1. Influence of TB in the detection of malignancies in oro and or pharyngeal lesions
2. Its performance in high risk groups.
3. Early identification of malignant changes in premalignant lesions.
4. As a method of follow-up. ⁷

Properties

Chemically, toluidine blue is referred to as tolonium chloride. Its molecular weight is 270.374 g/mol. It is soluble in water (up to 3.5%) and in alcohol (up to 0.5%)⁸ It is an acidophilic dye of the thiazine group that selectively stains acidic tissue components (carboxylates, sulfates, and phosphate radicals) such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)⁹⁻¹⁰. It has the staining property of metachromasia, which is due to the presence of repetitive phosphate groups in the nucleic acids and is dependent on temperature and the pH. The recommended pH is 6.0-7.0. The temperature should not exceed 30°C above which the metachromatic property diminishes in strength.

Principle

Toluidine blue is based on the principle of metachromasia⁸. It binds with the nucleohistones in the DNA by two ways.

1. Intercalation
2. Aggregation or stacking

The dye reacts with the tissues to produce a color different from that of the original dye and from the rest of the tissue. It is an event whereby a dye may absorb light at different wavelengths depending on its concentration and surroundings of the tissue and it has an ability to change its color without changing its chemical property. The physical changes that bring about this color change are a specialized, orderly form of dye aggregation. For metachromasia to occur there must be free electronegative groups on the surface of tissues. Its use *in vivo* is based on the fact that dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissues, shows loss of cell cohesion and increased mitosis.^{9,10,11} In addition, malignant epithelium contains wider intracellular canal which facilitates the penetration of dye. In

normal epithelium, the stain is lost after the application of 1% of acetic acid. But in benign ulceration condition, shows well defined uptake of dye at the because of the consequence of traumatic lesions and other margins whereas diffuse marginal pattern is characteristic of dysplasia or malignancy.¹¹

Technique of staining

TB can be used in two ways. It is either applied to the site of the lesion with a cotton applicator or it is used as mouth rinse. The procedure of staining is as follows:

- Oral examination
- Rinsing the mouth twice with water for 20 secs to remove the debris
- Application of 1% acetic acid for 20 secs to remove any ropey saliva
- Application of 1% TB solution for 20 secs either with cotton swab when a mucosal lesion is seen or given as a rinse
- Application of 1% acetic acid to reduce the extent of mechanically retained stain
- Rinsing oral cavity with water
- Oral examination and recording of the stained areas.

Interpretation

A dark blue (royal or navy) stain of either the entire lesion or a portion of it is considered as positive stain, lack of color absorption by the lesion as negative stain, and light or pale blue staining as doubtful. These cases are usually due to mechanical surface retention or inadequate removal of the stain.^{8,12,13} Mashberg suggests some areas not to be considered positive if it retains stain. These areas include the nucleated scales covering the papillae on the dorsum of the tongue, pores of seromucinous glands in the hard palate, dental plaques, gingival margins around each tooth, diffuse stain of soft palate transferred from the retained stain on dorsum of tongue, and ulceration lesions.^{5,12,13} Confusion obtain over the interpretation of pale colored staining. It is used as an adjunctive aid in the detection of premalignant and malignant lesions, in selecting biopsy site, in the screening of second primaries of the oral cavity, for the detection of multicentric tumors, in obtaining the marginal control of carcinoma, and during the followup of treated lesions.^{8,9,10,14}

Methylene blue (MB)

The procedure of MB staining was originally described by Japanese investigators for correcting the diagnosis of early gastric cancer. Its application has been reported recently in detecting some gastrointestinal abnormalities such as Barrett's esophagus, gastric cancer, prostate cancer, and also bladder cancer. However, its technique in detecting oral lesions by far is constrained.

Indications

1. Early evaluation of oral cancer and precancerous lesions.
2. used for intraoperative detection of canal isthmuses in molars during endoscopic periradicular surgery and to determine the areas of incomplete excision during peripheral osteotomy of aggressive lesions like odontogenic keratocyst (OKC) and ameloblastoma. This method has been justify to ensure complete removal of the lesion and hence decrease in the recurrence.^{15,16}

Lugol's iodine

Lugol's iodine, also known as Lugol's solution. Lugol's solution consists of iodine and potassium iodide. Earlier, Lugol's iodine has been used for evaluating cervical and esophageal epithelium. During colposcopic examination of uterine cervix, Lugol's iodine is applied to determine dysplastic epithelium and this test is called as Schiller's test.^{9,17}

Acetowhite staining

Acetic acid staining has been used as a part of colposcopic examination since 1938.¹⁵ Main functions to remove the ropey saliva and to reduce the extent of mechanically retained stain.¹⁸ Since it is relatively inexpensive and easy to use, interest has proceed in using acetic acid alone in the

evaluation of premalignant and malignant lesions.

Double staining

combination of two dyes to aid in the evaluation of oral malignant diseases. TB and Lugol's iodine combination has been used by Epstien, *et al.*, and Nagaraju, *et al.*, for the assessment of oral malignant diseases.^{9,19} TB will stain the abnormal epithelium, whereas Lugol's solution binds to glycogen present in the normal epithelium. The use of Lugol's iodine may be limited on lesions arising from keratinizing mucosa. Thus, the use of both tissue stains Zhu, *et al.*, have studied the combination of MB and Lugol's iodine double staining in the identification of esophageal carcinoma. The basis of the mucosal double staining method was that MB stains lesion blue and Lugol's iodine reversibly stains glycogen brown. Normal squamous epithelium appears unstained because it does not absorb MB, but in abnormal mucosa the superficial epithelium is often stained blue because it absorbs MB. Therefore, the area stained blue indicates the presence of carcinoma, the area stained brown belongs to normal squamous mucosa and the area between both the colors clarifies the invasive lesion of carcinoma.²⁰ can overcome this potential constraints of Lugol's iodine.

CONCLUSION

Toluidine blue staining is very useful in the developing countries like India because simple, economical, widely available, noninvasive, and easy to use. Supravital staining with 1% toluidine blue is useful in the early detection of malignancies. TB stain is of value due to its high sensitivity but is reduced in specificity due to the potential of false positive results in benign lesions. It is useful in high risk populations to enable earlier detection. It assists in selecting the best site for biopsy.

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