ORIGINAL RESEARCH

Assessment of Oral Mucosa in High Risk and Precancer Using Chemiluminescent Illumination and Toluidine Blue Supravital Staining

Anjana Ramanathan[1], Deepak T.A[2], Manjunath M[3], Sowmya Krishna[4], Annaji A.G[5]

1- Post Graduate, Department of Oral Medicine and Radiology, Vokkaligara Sangha Dental College and Hospital, Bangalore. 2- Professor, Department of Oral Medicine and Radiology, Vokkaligara Sangha Dental College and Hospital, Bangalore. 3- Professor and Head, Department of Oral Medicine and Radiology, Vokkaligara Sangha Dental College and Hospital, Bangalore. 4,5- Reader, Department of Oral Medicine and Radiology, Vokkaligara Sangha Dental College and Hospital, Bangalore.

Correspondence to:
Dr. Anjana Ramanathan, P.G., Department of Oral Medicine and Radiology, Vokkaligara Sangha Dental College and Hospital, Bangalore.
Contact Us: www.ijohmr.com

ABSTRACT

Introduction: Advancements in the field of oral cancer research have led to the development of diagnostic tools at both clinical and molecular level for the early detection of oral cancer. **Aims:** To assess and compare the efficacy of chemiluminescence and toluidine blue staining in the diagnosis in high-risk patients and precancer. **Materials And Methods**: Study involved two group of patients: group I included 10 patients with high risk for developing potentially malignant lesion. Group II included 10 patients with clinically discernible potentially malignant disorders. Complete oral examination was performed for each patient, followed by toluidine blue staining and Vizilite chemiluminiscence. Incisional biopsy was subsequently performed for the patients in who the tests gave positive results. **Results:** Sensitivity of Toluidine blue was 55.5%, and Specificity was 91.6%. Vizilite had sensitivity 88.8% and specificity 54.5%. The overall accuracy as per this study was calculated to be 80% for Toluidine blue and 70% for Vizilite. **Conclusion:** Vizilite has higher sensitivity, but poor specificity and Toluidine blue has very high specificity but lower sensitivity. Overall, accuracy of Toluidine blue was higher.

KEYWORDS: Diagnosis, Toluidine blue, Chemiluminiscence, Precancerous lesions

INTRODUCTION

Oral cancer is a major problem in the Indian subcontinent where it ranks among the top three types of cancer in the country[1]. Though the mortality is comparatively lesser than other cancers, its morbidity is significant with more impact on the psychological perspective. There are many proposed etiologies for oral cancer, of which tobacco abuse is of prime concern.

In 1978, the World Health Organization proclaimed that clinical presentations of the oral cavity that are recognized as potentially malignant disorders be classified into two broad groups, like lesions and conditions, with the following definitions:

A precancerous lesion is 'a morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart. Example: Leukoplakia.

A precancerous condition is 'a generalized state associated with a significantly increased risk of cancer.'[2] Example: Oral submucous fibrosis.

Although COE (conventional oral examination) may be effective as a screening test, there are still many problems with this approach. First, approximately 5–15% of the general population have oral mucosal abnormalities. Without question, the vast majority of these lesions are clinically/biologically benign. Also, it cannot discriminate Non progressive leukoplakia from its progressive counterparts.[3]

Till date, the proposed 'gold standard' for the diagnostic confirmation of dysplasia is assuredly histopathological examination. However, as scalpel biopsy is an invasive procedure with the disadvantage of tumor seeding, it becomes indicated only when the lesion displays either symptoms or clinical features of malignancy. Meanwhile, many of the innocuous and early stage oral cancerous lesions are observed clinically and left undiagnosed.[4] Thus various adjunctive and noninvasive tools have been developed at both the clinical and molecular level to assess the oral precancerous lesions. Here we shall discuss in detail, the diagnostic tool used for this study - Vizilite and Toluidine blue staining.

Bioluminescence, for the first time, was reported as far back as 1500 BC, in Chinese literature, and the best-known examples were emission of light from fireflies and glow-worms.

The term ''chemiluminescence'' was coined by Eilhardt Weidemann in 1888. It refers to the process of emission

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of light from a chemical reaction. Chemiluminescent reactions may occur in the gas, solid and liquid state. Its simplest representation being:

$$A + B = [I]^* \longrightarrow \text{products} + \text{light}$$

where [I]* is a highly energetic intermediate compound, which is produced from a chemical activation reaction when two reagents, namely 'A' and 'B' are mixed. The intermediate has a short life and returns to a lower energy state by emitting visible light. [4] The duration of these reactions can last from a second to more than a day. Chemiluminescent reactions can emit light of varying degrees of intensity and life, with colors that span the entire visible spectrum.

Vizilite is a recently introduced diagnostic tool devised for the simple and early diagnosis of oral cancer and is based on the principle of chemiluminescence. It has been shown to be an easy, quick, safe and non-invasive technique that is capable of detecting even the early, asymptomatic precancerous and cancerous lesions in the oral cavity. [5] Vital staining is the process of dyeing of the living cells or tissues. The staining succeeds in revealing the unapparent cytological details. Tolonium chloride (Toluidine blue) was introduced by Abbot laboratories and has been used as a vital stain to disclose dysplasia and carcinoma in situ of uterine cervix.

The objectives of this study were to assess and compare the effectiveness of chemiluminescent visualization and toluidine blue supravital staining in detecting lesions among patients with no clinically identifiable lesions and to assess its sensitivity and specificity in detecting the dysplastic changes in those patients presenting with clinically identifiable oral potentially malignant disorders.

MATERIALS AND METHODS

A total of 20 patients (males-18, females- 2) were selected for the study.

Inclusion Criteria

1. Patients with the habit of tobacco use in any forms irrespective of age, sex or socio-economic background.
2. Patients diagnosed clinically as having leukoplakia, Erythroplakia, Oral Submucous fibrosis or Oral lichen planus.

Exclusion Criteria

1. Patients without any habits of tobacco usage.
2. Patients with any systemic disorders or mentally unstable patients, unable to cooperate
3. Patients with dentures, sharp teeth and with parafunctional habits.

This study was done in 2 groups. Each group consists of 10 cases. Group I consists of normal appearing mucosa in patients who give a current history of tobacco usage. Group II consists of clinically diagnosed potentially malignant disorders. The patient consent was obtained. A detailed case history was recorded. Chemiluminescent illumination, Toluidine blue supravital staining were performed in all cases. Incisional biopsy was done in test-"positive" lesions.

Chemiluminescent illumination (Vizilite): The subjects in the study were instructed to rinse their mouth with the Vizilite rinse (1% acetic acid solution). They were asked to swish the rinse, all around the mouth for a total of one minute and expectorate it. The examination room illumination was dimmed to minimize the ambient light. The Vizilite capsule was activated by sharp bending, and assembled within the Vizilite retractor. The oral cavity was re-examined, now using the illumination from the Vizilite assembly. The observations were recorded accordingly and photographed. The presence of a characteristic "acetowhite" lesion after a one minute mouth rinse with 1% acetic acid solution was considered to be a "positive" result [Figure 1,2]. The absence of such findings was considered a "negative" result.

Figure 1: Vizilite illumination of malignant ulcer on lateral border of tongue reflecting "aceto-white" light.

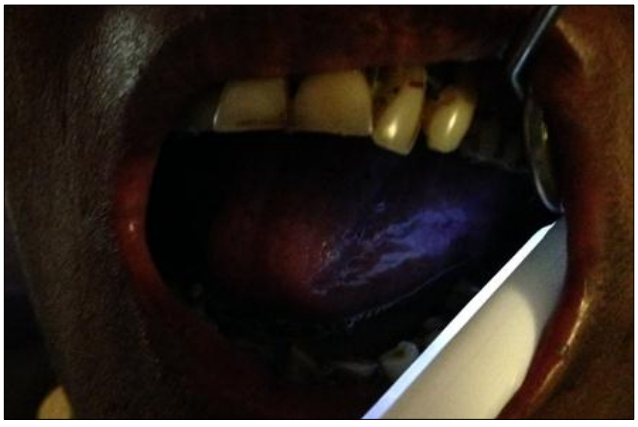

Figure 2: Vizilite illumination of the same lesion shows wider area of reflected bright white light

Toluidine blue staining: The intraoral lesion was mopped with 1% acetic acid. A cotton applicator tip was soaked with toluidine blue and used to apply the dye over the same lesion area for 30 seconds. 1% acetic acid on a cotton applicator tip was used again, to remove excess stain from the lesion. The observations were recorded in detail, and lesion was photographed. Lesions that

exhibited dark blue were considered as a "positive" test [Figure 3,4]/ while those that stained lightly or not at all were considered as “negative" test.

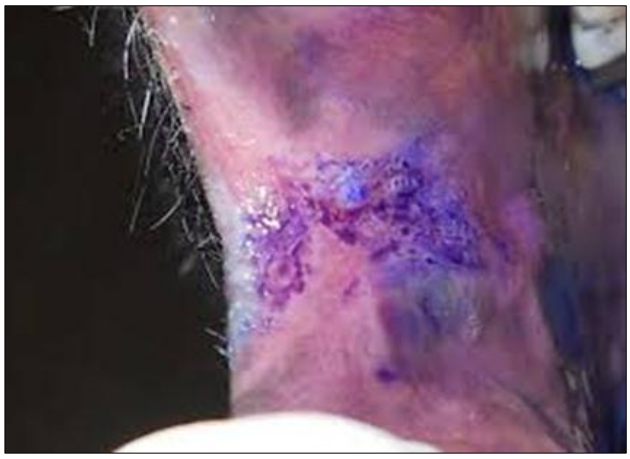

Figure 3: Toluidine blue staining of a leukoplakia lesion with areas of dysplasia at corner of mouth

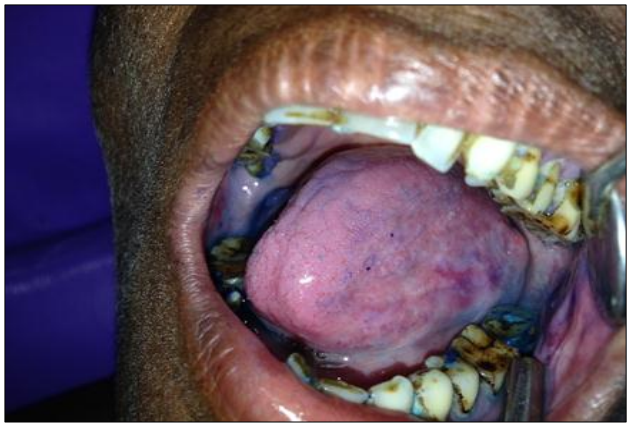

Figure 4: Clinical diagnosis of Erythroplakia on left lateral border of tongue stained with toluidine blue shows dark stain retention (dysplastic area) in center of lesion.

Ethics: The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975 that was revised in 2000.

RESULTS

Group I: 4 were Vizilite positive, 1 was toluidine positive. 2 out of the 4 positives showed mild epithelial dysplasia. Other 2 Vizilite positive the one toluidine blue positive showed normal epithelium[Table 1].

HISTOPATHOLOGY	VIZILITE POSITIVITY	TOLUIDINE BLUE POSITIVITY
Mild dysplasia (n=2)	2	0
Normal epithelium (n=8)	2	1

TABLE 1

Group II: 9 were Vizilite positive, and 5 were toluidine positive. 5 toluidine-positives showed epithelial dysplasia, 2 out of 5 toluidine negative showed mild dysplasia of which one was Vizilite negative. 3 showed normal epithelium[Table 2].

HISTOPATHOLOGY	VIZILITE POSITIVITY	TOLUIDINE BLUE POSITIVITY
Moderate dysplasia (n=5)	5.	5
Mild dysplasia (n=2)	1	0
Normal epithelium (n=3)	3	0

TABLE 2

DISCUSSION

Oral cancer is a treatable disease when it is detected early. Morbidity and mortality associated with oral cancer and its treatment can be significantly reduced if the delay in diagnosis is arrested Vizilite equipment was developed by the Trylon Corporation and was FDA approved for use in 2001 for testing people "at increased risk" for oral cancer.[6]

Vizilite is a painless, effective and fast procedure. The Vizilite kit comprises of a 1% acetic acid solution, a capsule (to be activated), a retractor and the manufacturer’s manual of instructions. The capsule is structured with an outer shell of flexible plastic and an inner vial of fragile glass. To activate it, the capsule must be bent for breaking the glass vial, so that the chemical products react inside and produce a bluish-white light with a wave length of 430-580nm, which can last for as long as 10 min. [5]

Mode Of Action: The intensity of the focused light is dimmed, and a diffuse, blue-white chemiluminescent light is produced which show the normal cells that absorb the light, and are depicted in the same bluish color. The abnormal cells, with a high nucleus- cytoplasm ratio, reflect the light back. Also the epithelium with excess keratinization, hyperparakeratinization and a predominant inflammatory infiltrate, appear aceto-white, with increased brightness and distinguished borders.[5]

Vizilite is known to have high sensitivity and low specificity. Vizilite has the advantage in that it is capable of delineating the sharp borders between normal and abnormal oral mucosa. The lesional borders defined by Vizilite, did not always coincide with their clinical outlines viewed under dental light and often extended beyond the clinically recognized outline.[7]

Toluidine blue (also known as tolonium chloride) is an acidophilic metachromatic dye that selectively stains acidic tissue components. Toluidine Blue in known to detect relative, rather than absolute differences, between normal and malignant cells and tissue. They can be used as 1% or 2% oral rinse or an application either in aqueous form or as weak acid solution.[8] Only about 5% of dye by weight is retained in oral cavity following expectoration. The preferential binding of toluidine blue to dysplatic tissue, rather than to normal epithelium is due to the

increased membrane permeability of these cells, which allows the stain to pass into them. Its intracellular binding is enhanced by the increased nuclear-cytoplasmic ratio of dysplastic cells.[9]

Principle:There are many systems of chemiluminescence of which the two most widely used are the luminol-based and the peroxy-oxalate based systems. The Vizilite capsule consists of an outer shell of flexible plastic cap that contains aspirin or acetyl salicylic acid and an inner fragile glass vial that contains hydrogen peroxide. Activation of the capsule, by flexing it, results in the inner fragile glass vial breaking to release the hydrogen peroxide. The two chemicals in the Vizilite capsule, i.e. acetyl salicylic acid and hydrogen peroxide react, and thereby release energy. A fluorescent dye accepts this energy and converts it into light. The color of the fluorescent dye determines the resulting color of the capsule when the chemical solutions are mixed. The basic principle of the reaction is that the reaction between the two chemicals releases enough energy to excite the electrons in this fluorescent dye. This causes the electrons to jump to a higher energy level and then fall back down and as a result, release light. Specifically, the hydrogen peroxide oxidizes the phenyl oxalate ester, to form phenol and an unstable peroxyacid ester. The unstable peroxyacid ester decomposes, resulting in the formation of phenol and a cyclic peroxy compound. This cyclic peroxy compound decomposes to carbon dioxide. This decomposition reaction releases the energy that excites the dye.[10]

Vital staining of the oral epithelium has been suggested as a means of surveillance in patients who are at a risk of developing oral cancer and for those who had confirmed neoplasms of other parts of aerodigestive tract.[8] Toluidine Blue has been used as a vital stain to delineate potentially malignant oral lesions and may succeed in identifying early lesions, which could be missed out on regular clinical examination. It is useful in obtaining the marginal control of carcinoma and in selecting the biopsy sample site in premalignant lesions.[8] Loss of heterozygosity may be detected in toluidine blue-stained lesions. Toluidine blue-stained tissue may appear dark royal blue or pale royal blue color.

Principle: Toluidine blue stains tissues based on the principle of metachromasia. The dye reacts with the tissues to produce a color different from that of the original dye and from the rest of the tissue. [7] Metachromasia is important as it is highly selective and only certain tissue structures can stain metachromatically.

The exact mechanism of action of toluidine blue staining has been controversial. The nuclei of inflammatory and cancer cells stain dark blue and the cytoplasm a very faint blue.

In the ultrastructural level, the dye tends to show an affinity for the perinuclear cisternae of DNA and RNA. Also, electron dense deposits notably fill the intercellular spaces of the tumoral lobules, and cover the nuclei of the inflammatory and cancer cells.

Number of false negatives is the chief indicator of the efficacy of a diagnostic test as it prevents rendering of any treatment protocols that can have a significant bearing on the survival of patients.[11] The high number of false positives may lead to overtreatment and adversely affects the the mental status of the patient, and his psyche based on the underlying fear of having cancer.

The main disadvantage of toluidine blue is that it gives information only about the surface changes.[11]The depth of the lesion cannot be assessed as it has been shown that toluidine blue stains to a depth of only two to ten cell layers.[12] A thick layer of keratin present in homogenous leukoplakia prevents the penetration of the toluidine blue dye, resulting in false negative test.

Evidence exists, to support that Vizilite enhances visual lesions to about 60%, identifying all the lesions which were previously detected by standard light, but no additional lesions.[8]

In the present study, of the 10 patients with clinically diagnosed premalignant lesions, 4 were homogenous leukoplakia, 1 non homogenous leukoplakia, 2 erosive lesions, 1 was reticular lichen planus, and 2 were Grade III OSMF. The biopsy was preferably done from the sites where both Vizilite and toluidine blue were positive. In the cases where either one came positive, that site was chosen for biopsy.

CONCLUSION

Chemiluminescence test was sensitive for potentially malignant lesions, high-risk patients, keratotic lesions and red-white lesions. It showed negative for erosive lesions. Toluidine blue staining test was fairly accurate in potentially malignant lesions, which presented as erosive and red-white lesions. It was negative for keratotic lesions.

Vizilite has a higher sensitivity but lower specificity while Toluidine blue, inspite of a lower sensitivity has a very high specificity. This makes it a highly reliable marker to proceed for biopsy procedures. The purpose of development of more diagnostic aids for early detection that are easy to use with the added benefit of ease of demonstration to the patient will be highly beneficial in our country, which brings us to the need for Vizilite and more such visually demonstrable diagnostic aids.

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