

Chemiluminescence and Toluidine Blue as Diagnostic Tools for Detecting Early Stages of Oral Cancer: An *invivo* Study

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ABSTRACT

Background: There is a need for development and use of diagnostic aids that help the dental specialist more readily identify and assess Potentially Malignant Epithelial Lesions (PMELs) and Oral Squamous Cell Carcinoma (OSCC). This study was done to assess the value of two such commercially available tools: chemiluminescent light kit or ViziLite and 1% toluidine blue.

Aims and Objectives: a) To detect epithelial dysplastic changes using chemiluminescence (commercially available as ViziLite) and toluidine blue staining in PMELs and OSCC patients and compare the results obtained with histopathological examination. b) To determine whether these techniques can be used to detect early epithelial dysplastic changes in clinically normal appearing oral mucosa of high risk (with habits) patients.

Materials and Methods: A total of 60 patients- 25 patients with PMELs, specifically oral leukoplakia, 10 patients with clinically diagnosed OSCC and 25 high risk patients with no clinically visible lesion, were screened with ViziLite and toluidine blue staining; followed by incisional biopsy.

Results: Sensitivity and specificity of ViziLite were calculated to be 95.45% and 84.6% respectively. ViziLite detected early epithelial dysplastic changes in one high risk patient with clinically normal appearing oral mucosa. Sensitivity and specificity of toluidine blue were calculated to be 86.36% and 76.9% respectively.

Conclusion: ViziLite was relatively reliable in screening PMELs compared to toluidine blue, and was a useful chair side diagnostic aid.

Keywords: Chemiluminescence, ViziLite, Toluidine blue, OSCC, PMELs

INTRODUCTION

'Oral cancer' is traditionally defined as squamous cell carcinoma of the lip, oral cavity and oropharynx [1]. Although representing 4% of the malignancies in the developed countries, Oral Squamous Cell Carcinoma (OSCC) accounts for almost 40% of all carcinomas in the Southeast Asia [2]. OSCC can be preceded by Potentially Malignant Epithelial Lesions (PMELs) which are clinically evident as erythroplakia or leukoplakia or lichen planus or actinic cheilitis. Other terminologies in use are 'atypia' and 'dysplasia', which denote the cellular changes occurring in the individual cell or in the epithelium as general [3].

Despite numerous advances in treatment of OSCC, the 5-year survival has remained approximately 50% for the last 50 years. This poor prognosis is likely due to the advanced extent of the disease at the time of diagnosis, with over 60% of patients presenting in stages III and IV [1]. An approach to this problem is to improve the ability of oral health care professionals to detect relevant PMELs at their earliest or most incipient stages. Such a goal can be achieved by increasing public awareness about the relevance of regular oral screening or case finding examinations to identify small, otherwise asymptomatic precancerous and cancerous lesions (secondary prevention). Another strategy is the development and use of diagnostic aids that help the dental specialist more readily identify or assess persistent oral lesions of uncertain biologic significance [1,4,5].

Many adjunctive techniques have emerged with claims of enhancing oral mucosal examinations and facilitating the detection of and distinctions between oral benign and oral premalignant and malignant lesions [5,6]. The objective of this study was to assess the value of commercially available chemiluminescent light kit or ViziLite

and 1% toluidine blue; as diagnostic aids in the early detection of oral cancer and PMELs.

Chemiluminescence (commercially available as ViziLite) is an oral examination screening aid that is claimed to improve identification, evaluation and monitoring of oral mucosal abnormalities in those with increased risk of oral cancer [7-10]. The principle of chemiluminescence has been employed in the field of Obstetrics and Gynaecology for the early detection of cervical cancer and pre cancer. The technique herein is referred to as Magnified Chemiluminescent visual Examination (MCE). Commercially available kit called Speculite is used to examine the cervix and vagina [11].

Chemiluminescence is the emission of light with limited emission of heat (luminescence), as the result of a chemical reaction [12]. The various colours produced are Blue, Green, Yellow-green, Yellow, Orange and Red [13]. There are many systems of chemiluminescence of which the two most widely used are luminol based and the peroxy-oxalate based systems [14,15]. ViziLite used in the present study was most likely based on peroxy-oxalate system [7]. It has an outer flexible capsule containing acetyl salicylic acid and inner fragile glass vial containing hydrogen peroxide. These chemicals react to produce a light of blue-white colour with wavelength between 430-580 nm [7,8].

The rationale behind chemiluminescence is that the application of acetic acid solution removes debris, disrupts the glycoprotein barrier on surface epithelium and dries the mucosa, allowing better penetration of light; hence oral mucosal abnormalities are better visualized due to changes in their refractive properties [16]. The blue white light produced is absorbed by the cells of the normal mucosa (with normal nuclear-cytoplasmic ratio i.e. 1:4); whereas reflected by the cells with abnormal nuclei including dysplastic and

neoplastic cells [7, 17-19]. Now the reason behind this reflection or scattering of light back from the neoplastic cells was explained by Rebekah Drezek et al., [20] who stated that the longer wavelength light is more transmitted while the shorter wavelength light is more reflected via scattering. The effect is most commonly observed in cells where the protein content increases. Now, when the cells mutate from normal to becoming cancerous, replication of nuclear DNA occurs at an accelerated rate, and DNA takes up a greater percentage of the total cell volume. The ratio between the nucleus and the cytoplasm increases until the nucleus takes up nearly 100% of the cell volume. Thus reflection of light occurs in cancerous cells.

Toluidine blue, an acidophilic metachromatic dye of thiazine group selectively stains acidic tissue components (sulfates, carboxylates and phosphate radicals), thus staining DNA and RNA [21-25]. Toluidine blue has been established as a diagnostic adjunct in detecting oral lesions related to invasive carcinomas, carcinoma in situ or early asymptomatic oral carcinomas [26-32].

Use of toluidine blue as a diagnostic aid for oral precancerous and cancerous lesions has been widely reported in the literature. But there is less information regarding the use of chemiluminescence. Hence, one of the aims of our study was to evaluate the efficacy of chemiluminescence as an oral cancer screening aid. This study is the first report of the use of a chemiluminescent light source (ViziLite) on clinically normal appearing oral mucosa of high risk (with habits) patients, to determine whether this technique can be used to detect early epithelial dysplastic changes.

MATERIALS AND METHODS

Study Sample

A total of 60 patients were selected from the out-patient department of Sibar Institute of Dental Sciences, Guntur, India, and study was conducted for a period of 24 months (2010-2011). The patients were divided into 3 groups: Group I (study group) consisted of 25 patients with PMELs, specifically oral leukoplakia. Group II (study group) consisted of 10 patients with clinically diagnosed OSCC. Group III (control group) consisted of 25 high risk patients with no clinically visible lesions in the oral cavity, but had chronic history of habits such as smoking, tobacco or betel quid chewing or alcohol consumption; or had undergone previous radiotherapy treatment for OSCC.

Diagnostic Kits

The ViziLite kit (Zila Manufacturer, USA) costed 40 \$, and contained 60 light sticks, ViziLite 1% acetic acid solution, capsule, retractor and

user instructions. ViziLite 1% acetic acid solution was composed of purified water, acetic acid, sodium benzoate, raspberry flavour and propylene glycol and alcohol base. The ViziLite capsule or chemiluminescent light stick was composed of an outer flexible plastic capsule containing salicylic acid and an inner fragile glass vial containing hydrogen peroxide. Activation of capsule was achieved by flexing it, wherein, the inner fragile glass ruptured releasing the hydrogen peroxide. The chemicals reacted to produce light of the blue-white colour with a wavelength ranging from 430 to 580 nanometres. The light lasted for approximately 10 minutes. 'Aceto-white' appearing areas on ViziLite examination were considered positive for the test. [Table/Fig-1,2] Normal mucosa gave 'blue-hue', which was considered negative for the test.

1% Toluidine blue solution (B-CHEMS, Chennai) was composed of tonium chloride-1 gram, acetic acid-10 ml, absolute alcohol-4.19 ml and distilled water-86 ml. Toluidine blue solution was applied with the help of cotton swab. 'Blue' retention of stain was considered as positive for the test. [Table/Fig-3] Area with no retention was considered negative for the test.

Based on the results of history and examination, patients were categorized under Group I, II or III.

Histopathological Examination

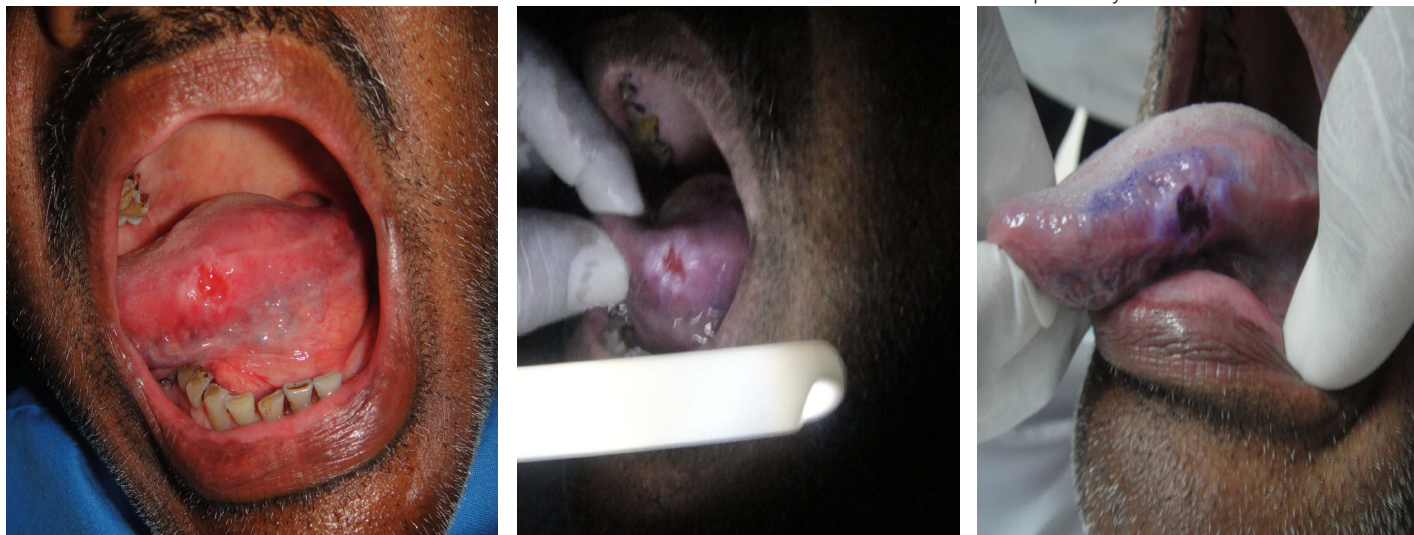
The examined lesions in Group I and II, whether positive or negative for these tests were subjected for incisional biopsy under local anaesthesia and specimens obtained were submitted for histopathological examination. Group III subjects with negative results in previous testing, and who had no oral mucosal abnormalities, were excluded from biopsy due to ethical reasons. But patients with positive results in both ViziLite and toluidine blue stain, were biopsied. Histopathological diagnosis of hyperkeratosis without dysplasia was considered a negative result and with dysplasia was considered a positive result [Table/Fig-4].

The histopathological findings were correlated with the results of other tests to determine the true positive, true negative, false positive, false negative, sensitivity and specificity values.

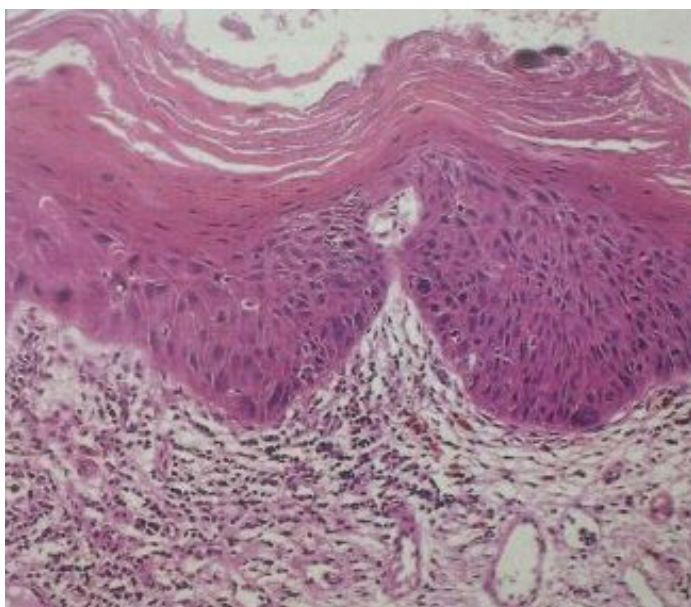
RESULTS

The data obtained was tabulated and subjected to statistical analysis. Group III was excluded from statistical analysis as histopathological examination was not performed. The tests employed for statistical analysis were Chi-square test and Kappa analysis.

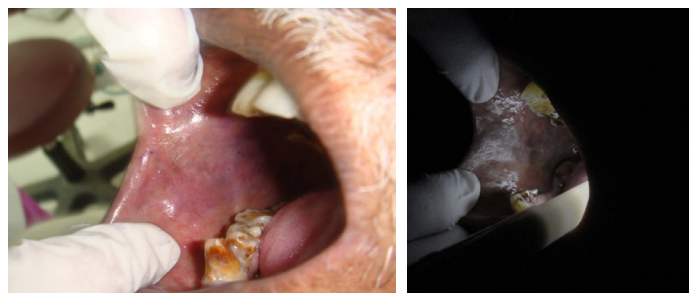
The results of ViziLite examination are tabulated in [Table/Fig-5]. P-value was found to be 0.000 and measure of agreement with Kappa analysis was .813. Sensitivity and specificity were calculated to be 95.45% and 84.6% respectively.



[Table/Fig-1]: Traumatic ulcer on lateral border of tongue, suspected of malignancy; **[Table/Fig-2]:** Aceto-white delineation of borders on ViziLite testing; **[Table/Fig-3]:** Retention of toluidine blue stain in the floor of the ulcer

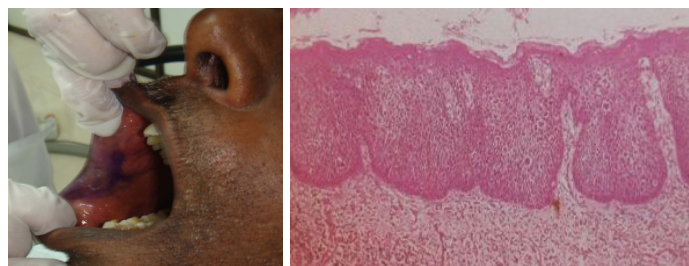


[Table/Fig-4]: Histopathological picture of Squamous Cell Carcinoma;



[Table/Fig-7]: No clinically visible lesion

[Table/Fig-8]: Aceto-white appearance seen on commissure of lip, on ViziLite testing



[Table/Fig-9]: Retention of toluidine blue stain in commissure of lip

[Table/Fig-10]: Histopathological picture of mild dysplasia

The results of Toluidine blue staining are tabulated in [Table/Fig-6]. P-value was found to be 0.009 and measure of agreement with Kappa analysis was .633. Sensitivity, specificity, positive predictive value and negative predictive value of toluidine blue were calculated to be 86.36%, 76.9%, 86.36% and 76.9% respectively.

			ViziLite		Total
			Negative	Positive	
Histopathology	Non dysplastic	Count	11	2	13
		% of total	31.4%	5.7%	37.1%
	Dysplastic	Count	1	21	22
		% of total	2.9%	60.0%	62.9%
Total		Count	12	23	35
		% of total	34.3%	65.7%	100.0%

[Table/Fig-5]: Results of ViziLite examination

			Toluidine Blue		Total
			Negative	Positive	
Histopathology	Non dysplastic	Count	10	3	13
		% of total	28.6%	8.6%	37.1%
	Dysplastic	Count	3	19	22
		% of total	8.6%	54.3%	62.9%
Total		Count	13	22	35
		% of total	37.1%	62.9%	100.0%

[Table/Fig-6]: Results of Toluidine blue staining

The Group III consisted of high risk patients but with clinically no visible lesions. Our study tried to rule out whether ViziLite could highlight or demarcate lesions in clinically normal appearing mucosa without doing invasive biopsy procedure. One patient with habits, but no clinically visible lesion revealed positive test for ViziLite and toluidine blue in the right commissure of lip [Table/Fig-7-10]. The area was biopsied and revealed dysplasia. Two of the patients who had undergone radiotherapy for previous OSCC revealed positive results with ViziLite and toluidine blue. Clinically no lesion was visible. These patients did not consent for biopsy. Three cases showed toluidine blue positivity in spite of normally appearing oral mucosa but were negative for ViziLite.

DISCUSSION

Our study results showed sensitivity of ViziLite of 95.45%, which was close to the results of studies done by Ram and Siar [7] and Camile S Farah et al., [9], where sensitivity was 100%. Study by Camile

S Farah et al., [9] proved that chemiluminescent light subjectively enhanced intra-oral visualization of all white lesions. Our results were not in accordance with the studies of Ravi Mehrotra et al., [19] where sensitivity was 0%. The reason was because ViziLite was unable to detect any true positive case out of four histopathologically positive cases. Authors have mentioned in their study that their limitation was that they did not classify lesions identified during the oral examination.

Our study results showed specificity of ViziLite of 84.6% which was not in accordance with other studies done by Ram and Siar [7], where specificity was 14.2% and Camile S Farah [9], where specificity was 0%. So the ViziLite in our study has showed better specificity in detecting true negative cases as compared to other studies. The reason for false positive cases in our study could have been due to reflection of chemiluminescent light because of surface keratinisation of oral mucosa which appeared acetowhite under chemiluminescent light.

On the basis of the results from our study, we came to a conclusion that ViziLite was more useful as an adjunctive diagnostic tool compared to toluidine blue, for identification of asymptomatic and clinically non-evident lesions, and for the follow-up and screening of previously treated cases of oral cancer. It was also capable of delineating the sharp borders between normal and abnormal oral mucosa. Furthermore, we observed that the lesional borders seen by ViziLite did not always coincide with their clinical outlines viewed under dental light, in the sense that they often extended beyond the clinically identified outline. This finding was best appreciated from photographic evaluation and not at the chair side.

Toluidine blue was reliable in detecting PMELs which present as erosive or ulcerated lesions, and it could give false positive results in keratotic lesions. The reason may be accounted to false retention of stain in ulcerated and inflamed areas of the lesion.

Toluidine blue had been proven to be effective in detecting satellite lesions (field cancerization) [33]. Our study is first reported one to detect whether chemiluminescence can detect early epithelial dysplastic changes in clinically normal appearing oral mucosa of high risk (with habits) patients. One patient with habits, but no clinically visible lesion revealed positive test for ViziLite and toluidine blue in the right commissure of lip and revealed dysplasia after histopathological examination. Two of the patients who had undergone radiotherapy for previous OSCC revealed positive results with ViziLite and toluidine blue. Now the reason behind these changes would be molecular. There may be abnormal molecular

changes which might be occurring prior to cellular changes and which could be impossible to detect even with histopathology. This focuses on the concept of "Field Cancerization", where abnormal molecular changes take place even in normal appearing oral mucosa adjacent to or to the contra lateral side. It is presumably caused by the consumption of tobacco and intake of alcohol in these patients. Patients with field cancerization may harbour patches of dysplastic or premalignant changes throughout the aero digestive tract. It is thought that these patches represent nascent cancers in the early stages of clinical presentation [34].

So from these findings, we concluded that we have to see every false positive case with suspicion even though the biopsy result is negative. Regular check-ups and follow ups of patients is mandatory.

CONCLUSION

Chemiluminescent light or ViziLite is useful as an adjunctive diagnostic tool for the detection of oral cancer and PMELs. However, well-controlled clinical trials are needed that specifically investigate the ability of chemiluminescence to detect precancerous lesions that are invisible by conventional oral examination alone. If such discrimination can be confirmed, it would support the use of this technology as a true screening device. Although major limitation of ViziLite is its high cost and the fact that it can be used only once for each patient.

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