

Clinical Paper
Head and Neck Oncology

Chemiluminescence as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions

S. Ram, C. H. Siar

Department of Oral Pathology, Oral Medicine & Periodontology, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

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Abstract. Chemiluminescence was evaluated as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions (PMELs) by comparing it against 1% toloum chloride mouth rinse. Forty-six clinically identified lesions [14 primary squamous cell carcinoma (SCC), 26 PMELs and 6 benign lesions] and five cases of normal oral mucosa from 40 subjects (inclusive of 10 previously treated SCC cases) were examined with a commercial chemiluminescent kit (Vizilite[®]) and toloum chloride. Biopsy and histological verification of 31 lesions disclosed 14 SCC (45.2%), 10 epithelial dysplasias (32.3%), 5 lichen planus (16.1%) and 2 benign lesions (6.4%). For the remaining 15 lesions, a biopsy was not performed owing to patient's lack of consent or ill-health. The five cases of normal oral mucosa which tested negative for both tools were also not biopsied for ethical reasons. Sensitivity for Vizilite[®] and toloum chloride was 100% and 70.3%, respectively; and specificity was 14.2% for Vizilite[®] and 25% for toloum chloride. Their accuracy was 80.6% and 64.5%, respectively. Current findings suggest that chemiluminescence is a more reliable diagnostic tool than toloum chloride in the detection of oral cancer and PMELs, and for follow-up of patients treated for the same.

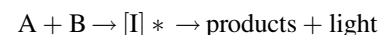
Key words: chemiluminescence; Vizilite[®]; toloum chloride; oral cancer; PMEL.

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Advancements in the field of oral cancer research have led to the development of diagnostic tools at both the clinical and molecular level for the early detection of oral cancer. Clinical diagnostic tools available for the early detection of oral cancer include toloum chloride or toluidine blue dye, Lugol's iodine, Oral CDx[®] brush biopsy kits, contact microscope (The Cyscope[™]) and Vizilite[®]¹⁵.

Luminescence in living organisms (bioluminescence) has been reported as far back as 1500 BC in the Chinese literature, the best-known examples being emission of light from fireflies and glow-worms. The first report of artificial luminescence (chemiluminescence) was in 1669 by a German physician, Henning Brand, who discovered phosphorus. The term "chemiluminescence" was first coined by Eilhardt Wei-

demann in 1888. Chemiluminescent reactions occur in the gas, solid and liquid state. In its simplest form it can be represented as:



where [I]^{*} is a highly energetic intermediate compound produced from a chemical activation reaction when

two reagents A and B are mixed. The intermediate is short-lived and returns to a lower energy state by emitting visible light. The reactions can last from a second to more than a day¹.

The term 'Chemiluminescence' refers to the emission of light from a chemical reaction. Chemiluminescent reactions emit light of varying degrees of intensity and lifetime, with colors that span the visible spectrum¹. Vizilite[®] is a recently introduced (commercially available) diagnostic tool devised for the early detection of oral cancer and is based on the principle of chemiluminescence. Apparently this is an easy, safe and non-invasive technique capable of detecting early asymptomatic precancerous and cancerous lesions in the oral cavity. However, thus far, there were no reports of clinical trials in the literature to substantiate these claims.

One of the earliest clinical diagnostic tools used for oral cancer detection is tolonium chloride or toluidine blue dye¹⁶. This dye has been used for about four decades by dentists for the purpose of detecting oral cancer^{7,19}. However, its acceptance as a potential oral cancer detection tool by the dental profession has on the whole been subdued due to wide-ranging reports on its sensitivity and specificity.

Oral cancer is the sixth most common malignancy worldwide. It remains a highly lethal and disfiguring disease⁷. The 5-year survival rate for oral cancer patients remains unchanged at 50% for the past five decades, despite improvements in surgical and radiation techniques as well as advancements in chemotherapy^{6,8,11,18,25}. At the time of diagnosis, the majority of lesions are found to be at Stage III, with more than 50% of these cases exhibiting metastatic lymphadenopathy. Patients generally do not seek treatment until the lesion is larger than 1 cm in size. However, when diagnosed at an early stage, oral cancer is often curable and inexpensive to treat¹⁹.

Dentists play an important role in the primary, secondary and tertiary prevention of oral cancer. Primary preventive measures such as changing habits and lifestyle are difficult and slow to implement. This is what makes the early detection of malignant and potentially malignant epithelial lesions (PMELs) through screening so important^{2,12}. The earlier these lesions are detected the greater the chance of recovery and a good quality of life and function^{3,8,11,12}. In the early stages, oral cancer is difficult to detect for either the patient or the dentist.

The primary method employed by dentists in the detection of oral cancer is a visual examination and palpation of the oral structures¹⁹. There is sufficient evidence that visual inspection alone is not adequate to differentiate early oral cancer from benign lesions regardless of the expertise of the clinician^{3,19}. 'Mirror-image' biopsies of normal-looking mucosa from patients with oral cancer and precancer involving the contralateral side revealed that 58% of these apparently normal-looking mucosa demonstrated abnormal histological findings ranging from reactive changes to frank microinvasive carcinoma²⁴. Moreover, in patients treated for previous upper aerodigestive tract cancer, clinical oral findings may be difficult to assess because persistent oral discomfort and mucosal changes after the primary therapy for the cancer may obscure or mimic suspicious tissue changes⁴. The use of a reliable diagnostic tool is therefore necessary to detect oral cancer at an early stage.

The objective of this study was to assess the value of a commercially available chemiluminescent light kit or Vizilite[®] over 1% tolonium chloride as a diagnostic aid in the early detection of oral cancer and PMELs.

Materials and methods

Diagnostic kits

Approval from the Research Ethics Committee, Faculty of Dentistry, University of Malaya, was obtained prior to the commencement of this study. The instructions conformed to the International Ethical Guidelines for biomedical research involving human subjects. The 1% tolonium chloride and 1% acetic acid rinses were compounded at the Research Laboratory, Faculty of Dentistry, University of Malaya, under the supervision of the Chief Pharmacist of the Medical Centre, University of Malaya, and in accordance with the recommendations of MASHBERG^{13,14}. A 100 ml of tolonium chloride was freshly prepared each time by mixing 1 g tolonium chloride with 10 ml acetic acid, 4.19 ml absolute alcohol and 86 ml of distilled water^{13,14} while a 100 ml of 1% acetic acid rinse was prepared by diluting 1 ml of glacial acetic acid with 99 ml distilled water. The Vizilite[®] kit, manufactured by Zila Pharmaceuticals, Phoenix, AZ, USA, consisted of a Vizilite[®] 1% acetic acid solution, capsule, retractor and user instructions. The contents of the Vizilite[®] 1% acetic acid solution are purified water, acetic acid, sodium benzoate, rasp-

berry flavour, and base of propylene glycol and alcohol. The Vizilite[®] capsule or chemiluminescent light stick comprises an outer flexible plastic capsule containing Aspirin or acetyl salicylic acid and an inner fragile glass vial containing hydrogen peroxide (personal communication). Activation of the capsule is achieved by flexing it, wherein, the inner fragile glass vial ruptures releasing the hydrogen peroxide. The chemicals react to produce light of the blue-white colour with a wavelength ranging from 430 to 580 nm. The light lasts for approximately 10 min.

Selection criteria

The subjects selected for this prospective study were individuals whose ages were 35 years and above, and presenting with either oral cancer or PMELs, or history of having undergone previous treatment for oral cancer or PMELs, or being suspected of having oral cancer or PMELs, or having a history of high risk habits such as smoking, tobacco or betel quid chewing or alcohol consumption. The presence of either one of the above factors or a combination of any of these factors formed the basis for the selection of subjects for this study.

Study sample

A total of 40 subjects (17 men and 23 women) comprising 5 Malays, 14 Chinese and 21 Indians were selected from the Departments of Oral Pathology, Oral Medicine & Periodontology, and Oral & Maxillofacial Surgery, Faculty of Dentistry, University of Malaya. These subjects were between the age groups of 35 and 80 years with a mean age of 56.75 years. Fourteen subjects (35%) were from the age group of 61 to 70 years. There were 27 subjects (11 men and 16 women; 4 Malays, 8 Chinese and 15 Indians) with either primary squamous cell carcinoma (SCC) or PMEL, and another 13 subjects (6 men and 7 women; 1 Malay, 6 Chinese and 6 Indians) with a history of having undergone previous surgical or radiation therapy for the above mentioned conditions.

Fourteen subjects (2 men and 12 women; 4 Malays, 5 Chinese and 5 Indians) had no history of habits. In the remaining 26 subjects, 13 (7 men and 6 women; 1 Malay, 4 Chinese and 8 Indians) had history of single habits which included cigarette-smoking (4 subjects), alcohol consumption (3 subjects) and betel quid chewing (6 subjects). The remaining 13 (8

men and 5 women; 4 Chinese and 9 Indians) had a history of multiple habits including cigarette-smoking and alcohol consumption (5 subjects), bidi smoking and alcohol consumption (1 subject), betel quid chewing with tobacco (5 subjects), betel quid chewing with tobacco and alcohol consumption (1 subject) and tobacco chewing with alcohol consumption (1 subject).

Screening procedure using Vizilite® and 1% tolonium chloride rinse

All subjects were briefed about the clinical procedure and purpose of the study, and patient consent was obtained prior to the screening procedure. A detailed case history was recorded and the screening was performed initially using Vizilite® (Table 1), followed by examination with 1% tolonium chloride (Table 2) as illustrated in Fig. 1A–E. Photographs were taken at every step of the procedure. The biopsy sites were selected based on the clinical appearance of the lesion(s) and the results of the Vizilite® and tolonium chloride examination. A total of 31 lesions (28 subjects), whether positive or negative for these tests, were subjected to incisional biopsy under local anaesthesia, and the specimens obtained were submitted for histological examination. In those subjects with multiple lesions, the site of biopsy was selected based on the clinical characteristics such as appearance, size and site of the lesion as well as the results of the diagnostic tools. In these instances, the clinically most suspicious sites were biopsied. Five subjects who had no oral mucosal abnormalities were tested negative for both diagnostic tools, and no biopsies were performed for ethical reasons. In another 7 subjects (15 lesions), a biopsy was not performed due to patients' ill-health or lack of consent. Five of these

Table 1. Screening procedure for detection of oral cancer and PMEL using Vizilite®

Conventional examination of the oral cavity using dental chair light
Record location, size, morphology and surface characteristics of lesion(s)
Photograph the lesion(s)
Rinse mouth with 30 ml of 1% acetic acid and expectorate after 1 min
Activate Vizilite® capsule and place it in the Vizilite® retractor
Dim surgery lights and examine the oral cavity
Record and photograph any findings
Rinse mouth with water and expectorate after 20 s

Table 2. Screening procedure for detection of oral cancer and PMEL using 1% tolonium chloride mouth rinse

Rinse mouth with 10 ml of 1% tolonium chloride and expectorate after 1 min
Rinse mouth with 10 ml of 1% acetic acid and expectorate after 20 s
Rinse mouth with water and expectorate after 20 s
Examine the oral cavity
Record and photograph any findings
Incisional biopsy under local anaesthesia

subjects were patients who had undergone radiotherapy for primary SCC, and now presenting with clinically suspicious lesions. The remaining two subjects did not consent for a biopsy although they consented for the screening part of the procedure. All these 15 lesions that were not histologically verified were excluded from evaluation for true/false positivity or negativity of these tests.

Histological tissue processing and analysis

The 31 biopsy specimens obtained were fixed in 10% formal saline, and processed at the Oral Pathology Laboratory, Faculty of Dentistry, University of Malaya. Five-micron thick sections were prepared and stained routinely with hematoxylin and eosin for microscopic examination blind. The histological findings were correlated with the clinical findings to determine the true positive, true negative, false positive, false negative, sensitivity, specificity and

accuracy values. The definitions for these values are as follow:

- *True positives* (TP): are those persons with the disease who generate a positive test.
- *True negatives* (TN): are those persons with a negative test result who do not have the disease.
- *False positives* (FP): are those persons with a positive test result who do not have the disease.
- *False negatives* (FN): are those persons with the disease who generate a negative test.
- *Sensitivity*: also known as the true-positive rate, is the proportion of diseased individuals (confirmed by the gold standard—biopsy) who are correctly identified by the test.
- *Specificity*: also known as the true-negative rate, is the proportion of non-diseased individuals (confirmed by the gold standard—scalpel biopsy) who are correctly identified by the test.



Fig. 1. (A)–(C) Oral cancer screening procedure for Vizilite®. (A) Vizilite® kit, (B) activation of Vizilite® capsule, (C) examination of patient with Vizilite®. (D) and (E) Oral cancer screening procedure for tolonium chloride. (D) 1% tolonium chloride and acetic acid rinses, (E) 1% tolonium chloride rinse.

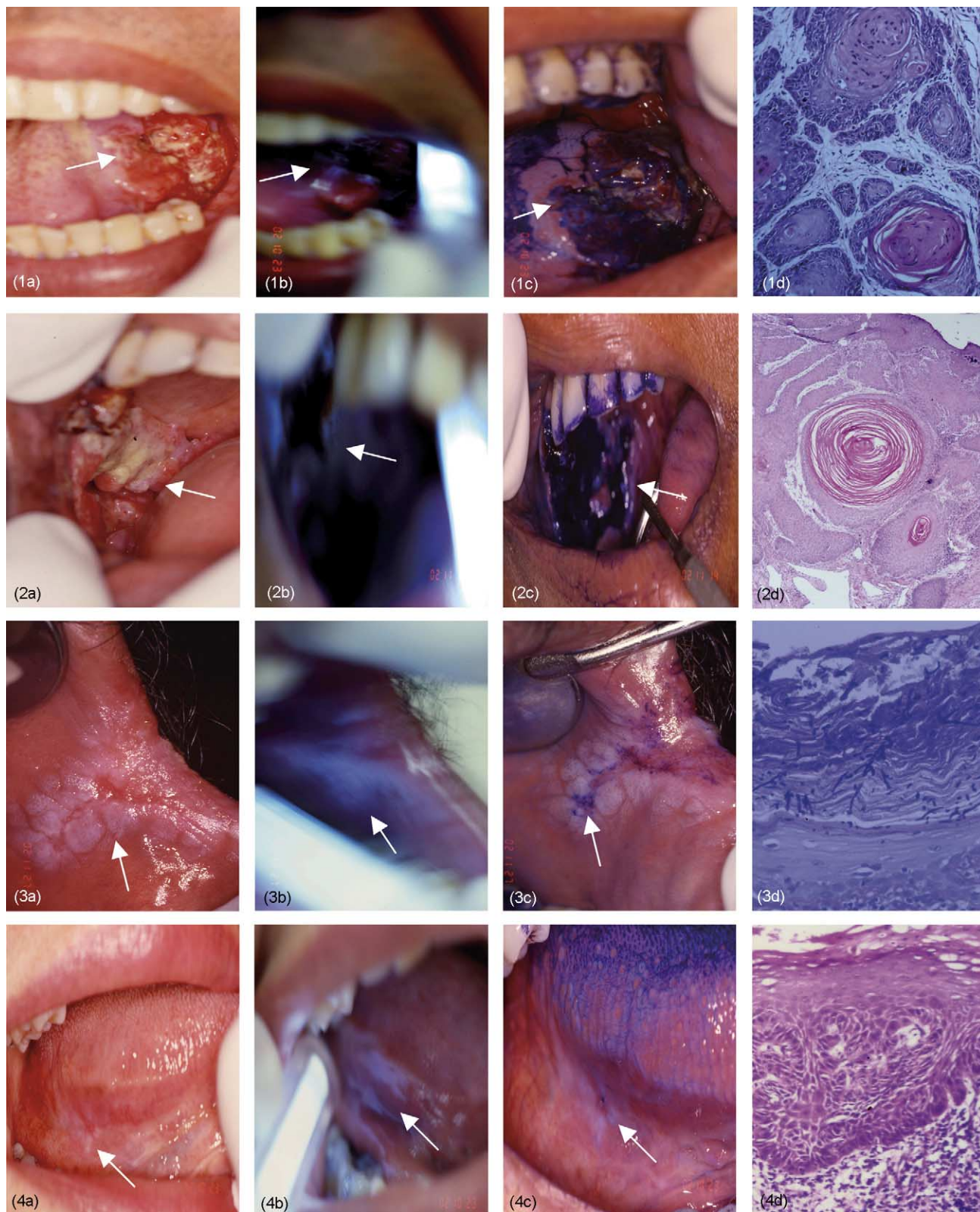


Fig. 2. Case 2.1: (1a) Clinically identified SCC left lateral posterior tongue (arrow). (1b) Vizilite[®] positive (arrow). (1c) Tolonium chloride positive (arrow). (1d) Histologically well-differentiated SCC (H&E, $\times 40$). Case 2.2: (2a) Clinically identified SCC right retromolar trigone (arrow). (2b) Vizilite[®] positive. Note that the Vizilite[®] lesional border differs from the clinical outline (arrow). (2c) Tolonium chloride positive (arrow). (2d) Histologically well-differentiated SCC (H&E, $\times 40$). Case 2.3: (3a) Clinically identified leukoplakia left commissure (arrow). (3b) Vizilite[®] positive. Note that the Vizilite[®] lesional border differs from the clinical outline (arrow). (3c) Tolonium chloride negative (arrow). (3d) Histologically chronic hyperplastic candidiasis (DPAS, $\times 200$). Case 2.4: (4a) Clinically non-evident lesion right lateral posterior tongue with only

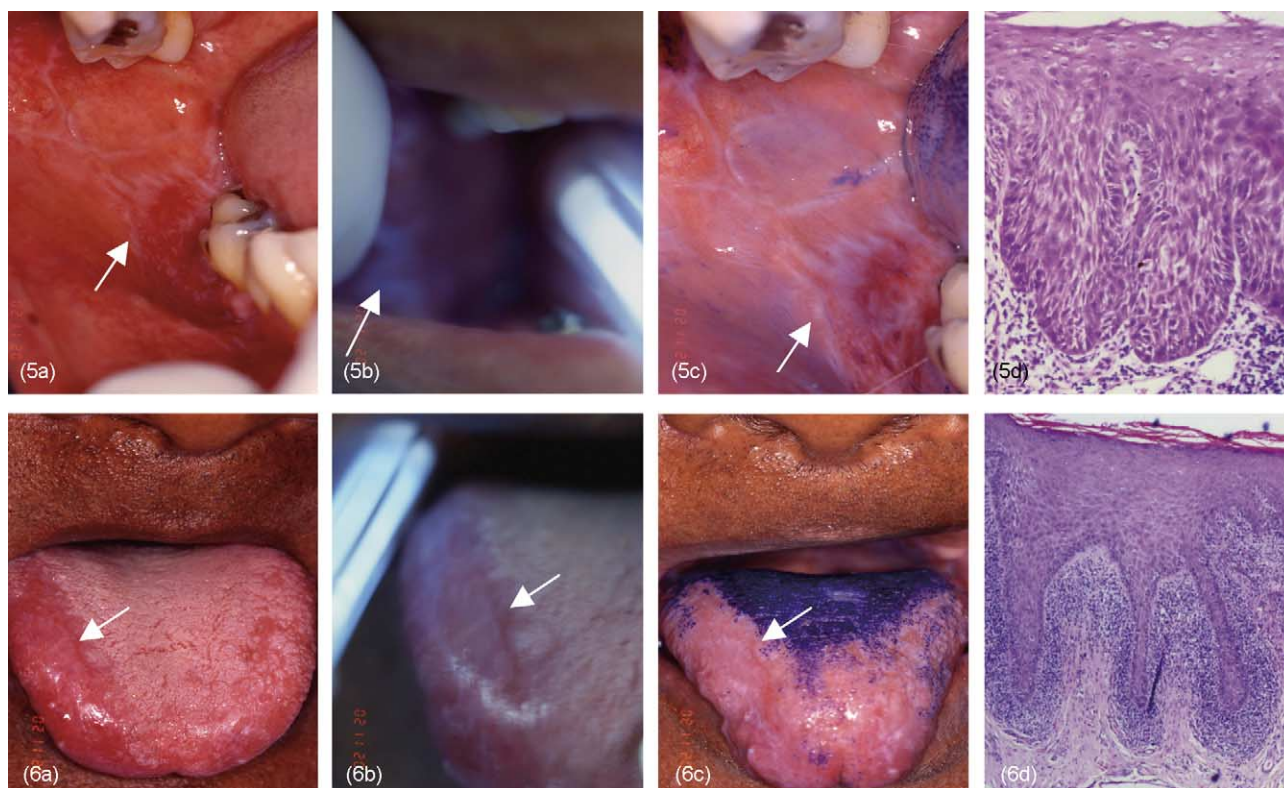


Fig. 2. (Continued).

- **Accuracy:** accuracy is a measure of the overall agreement between the diagnostic test and the gold standard (scalpel biopsy). The more accurate the test, the fewer false-negative and false-positive results.

Results

A total of 40 subjects were studied prospectively. Forty-six lesions in 35 subjects were identified clinically. Lesions that reflected the blue-white light were considered Vizilite[®] positive. Similarly, lesions that stained dark blue with tolonium chloride were considered as positive. Those that stained faintly or showed equivocal staining were interpreted as tolonium chloride negative. In five subjects the oral cavity showed no abnormalities and was categorized as negative for both Vizilite[®] and tolonium chloride.

During Vizilite[®] examination, the blue-white light was well reflected by the keratotic, atrophic and erythematous areas of

the lesions, delineating them from the surrounding normal mucosa. However, with tolonium chloride rinse, there was no or faint retention of the stain in most of the keratotic lesions ($n = 5$). In lesions with admixed keratotic, atrophic and erythematous areas as observed in erosive/atrophic lichen planus with superficial ulcerations ($n = 1$) or speckled leukoplakia ($n = 1$), the dye was retained only in the erythematous, erosive or atrophic areas of the affected mucosa. The intervening keratotic areas of the affected mucosa failed to retain the stain.

Thirty-one of 46 clinically identified lesions were biopsied and the correlative analysis between the histological diagnosis and diagnostic tool results is summarized in Table 3 and illustrated in Fig. 2 (Cases 1–6). Both Vizilite[®] and tolonium chloride were positive for the 14 clinically identified and histologically confirmed cases of SCC. Nine cases of clinically identified leukoplakias and one case of clinically identified radiation mucositis, upon biopsy and subsequent histological

examination were diagnosed as epithelial dysplasias. In general, all dysplasias (10/10) were identified using the Vizilite[®] but only half of these cases (5/10) were identified subsequently by the tolonium chloride rinse.

In this study, oral lichen planus was viewed as an immune-mediated chronic mucocutaneous disorder. All five clinically identified and histologically confirmed cases of oral lichen planus that were positive with Vizilite[®] were considered as false positives (Table 3). Only two out of the five lichen planus lesions were identified using tolonium chloride indicating two false positives for the dye.

The sensitivity, specificity and accuracy for Vizilite[®] and tolonium chloride are shown in Table 4. Vizilite[®] demonstrated no false negative results and therefore yielded 100% sensitivity in the detection of SCCs and epithelial dysplasias. Overall, the sensitivity for Vizilite[®] and tolonium chloride was 100% and 70.3%, respectively; and specificity was 14.2% for Vizilite[®] and 25% for tolonium chloride.

complaint of soreness by patient (arrow). (4b) Vizilite[®] identified lesion appearing as an irregular blue-white reflection (arrow). (4c) Tolonium chloride negative (arrow). (4d) Histologically moderate epithelial dysplasia (H&E, $\times 200$). Case 2.5: (5a) Clinically identified erosive lichen planus right retromolar trigone (arrow). (5b) Vizilite[®] positive (arrow). (5c) Tolonium chloride negative (arrow). (5d) Histologically severe epithelial dysplasia (H&E, $\times 200$). Case 2.6: (6a) Clinically identified atrophic lichen planus right lateral tongue (arrow). (6b) Vizilite[®] positive (arrow). (6c) Tolonium chloride negative (arrow). (6d) Histologically lichen planus (H&E, $\times 40$).

Table 3. Histopathological diagnosis of oral lesions and results

Histopathological diagnosis	N (%)	Vizilite®		Tolonium chloride	
		+	–	+	–
Squamous cell carcinoma	14 (45.2)	14	–	14	–
Epithelial dysplasia	10 (32.3)	10	–	5	5
Mild	6 (19.4)	6	–	2	4
Moderate	3 (9.7)	3	–	2	1
Severe	1 (3.2)	1	–	1	–
Lichen planus	5 (16.1)	5	–	2	3
Benign keratosis	2 (6.4)	1	1	1	1
Total	31 (100)	30	1	22	9

N: number of lesions; (%): percentage; +: positive; –: negative. Note: Five cases of normal oral mucosa tested negative for Vizilite® and tolonium chloride had no histopathological confirmation as biopsies were not performed due to ethical reasons.

Their accuracy was 80.6% and 64.5%, respectively (Table 4).

Discussion

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 is referred to as magnified chemiluminescent visual examination (MCE), wherein, Speculite®—a commercially manufactured kit—is used to examine the cervix and vagina. Following a 3–5% acetic acid wash, the cervix is examined using the low energy, diffuse, blue-white chemiluminescent light source with peak outputs near 430, 540 and 580 nm wavelengths^{10,17}. The MCE of the cervix with Speculite® was 90% sensitive in detecting biopsy proven carcinoma *in situ* (CIN) compared with 55% when the cervix was examined with the use of magnification and projected incandescent light^{9,10}.

There are many systems of chemiluminescence of which the two most widely used are the luminol based and the peroxy-oxalate based systems^{1,10}. Speculite® is based on the peroxy-oxalate system of chemiluminescence¹². The system of chemiluminescence that the Vizilite® kit is based on remains unknown. The manufacturer of Vizilite® claims that the blue-white light is absorbed by the cells of the normal mucosa and is reflected by cells with abnormal nuclei including dysplastic and neoplastic cells. The acetic acid rinse putatively removes debris and disrupts the glycoprotein barrier on the surface of the epithelium allowing pene-

tration of the light (Source: Zila Pharmaceuticals, Phoenix, AZ, USA).

This study is the first report assessing the value of a commercially manufactured chemiluminescent agent Vizilite® as a diagnostic aid in the detection of oral cancer and PMELs. Vizilite® has the advantage in that it is capable of delineating the sharp borders between normal and abnormal oral mucosa. A similar observation was reported with Speculite® in the early detection of cervical cancers and precancer¹⁷. Furthermore, we observed that the Vizilite® lesional borders 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 chairside.

In the current series of 14 SCC evaluated all were clinically obvious malignant lesions which could be recognized without the aid of adjunctive diagnostic tools. The reasons for screening these cases under Vizilite® are two-fold: (1) To determine the characteristics of clinically obvious SCC when visualized under chemiluminescent light, and (2) To screen for possibility of field cancer change in other parts of the apparently normal mucosa. In this respect, an asymptomatic white lesion each was identified on the ventrolateral surface of the tongue in two previously treated cases of oral SCC using chemiluminescent light, while tolonium chloride dye failed to detect the lesions. Subsequent histological examination showed them to be mild epithelial dysplasias. For the same aforementioned reasons

Vizilite® screening was performed on the nine cases of clinically identified leukoplakias and one case of clinically identified radiation mucositis. These lesions upon biopsy and subsequent histological examination were diagnosed as epithelial dysplasias. On the basis of these observations, Vizilite® proved to be more effective than tolonium chloride in the identification of asymptomatic and clinically non-evident lesions, and for the follow-up and screening of previously treated cases of oral cancer.

There were several limitations associated with the use of chemiluminescent light or Vizilite® as a diagnostic aid for the detection of oral cancer and PMELs. Vizilite® is expensive and can be used only once for each patient. As of 7 June 2004, six kits cost 169.95 United States of America dollars (USD) and 40 kits cost 980.00 USD. Therefore, the cost of a single kit ranges between 24.50 and 28.32 USD (Source: www.pattersondental.com). Although the Vizilite® or chemiluminescent light is superior to tolonium chloride in terms of sensitivity and accuracy, it fails to differentially identify biopsy sites unlike tolonium chloride. Moreover, in our study we have examined only overtly obvious cases of oral cancer which do not require special screening tests for identification. In this respect, we have not been able to detect any case of early oral cancer (CIN) in normal-looking oral mucosa with this tool. The other shortcoming is the observed false positive rate of 6/31 lesions indicating that Vizilite® is non-specific and likely to result in many unnecessary biopsies. In subjects with multifocal lesions ($n = 17$), following the screening protocol, we had to rely on clinical features such as size, appearance and location to determine the most appropriate lesion to be biopsied.

Tolonium chloride is an acidophilic or basic metachromatic dye that selectively stains the acidic tissue components, sulfate, carboxylate and phosphate radicals such as DNA and RNA^{20–22}. The exact mechanism of action of the dye is a subject of controversy. STRONG et al.²³ suggested that although the dye has affinity towards nuclei, much of its action is based on the fact that the haphazard arrangement of tumour cells creates intercellular spaces or canaliculi that allow penetration and

Table 4. Correlation of clinical and histopathological findings

Diagnostic tools	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	Accuracy (%)
Vizilite®	24	1	6	0	100	14.2	80.6
Tolonium chloride	19	1	3	8	70.3	25	64.5

(%): percentage.

retention of the dye. Although the normal mucosa does not stain macroscopically, light microscopic analysis revealed a slight uptake of the dye by the superficial cell layer⁵. In this reported study, two samples of infiltrative squamous cell carcinoma that were deeply stained macroscopically by the dye were analysed microscopically⁵. At the light microscopic level, the dye penetration was to a depth of 50 µm. The nuclei of inflammatory and cancer cells stained dark blue and the cytoplasm a very faint blue. On the ultra-structural level, the dye showed affinity for the perinuclear cisternae of DNA and RNA and electron dense deposits filled the intercellular spaces of the tumoral lobules and covered the nuclei of the inflammatory and cancer cells. From this study, HERLIN *et al.*⁵ observed that both the inflammatory and cancer cells accumulate the dye and therefore, the mechanism of action was based on the tissue and membrane permeability factor rather than cellular specificity.

Although Vizilite[®] and toluidine chloride identified all SCC in the present study, variable dye uptake was observed between exophytic and ulcerated SCC. The dye showed excellent retention and staining in the ulcerated lesions compared to the exophytic lesions owing to the increased intercellular spaces enabling better penetration of the dye. For the same reasons, this variable dye uptake was similarly observed in the 10 epithelial dysplasias examined.

In conclusion, chemiluminescent light or Vizilite[®] is useful as an adjunctive diagnostic tool for the detection of oral cancer and PMELs and follow-up of subjects treated for the same. However, further studies are required to evaluate the full potential of chemiluminescence or Vizilite[®] for target screening of high-risk group individuals.

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References

- CARLSON R, LEWIS SW, LIM KF. Seeing the light: using chemiluminescence to demonstrate chemical fundamentals. *Aust J Chem Ed* 2000; **14**: 51–53.
- CRAIG G, JOHNSON N. Oral cancer: guidelines for early detection. *BDA Occasional Paper BDA News* 1998; **5**: 5–19.
- EPHROS H, MASHBERG A. Toluidine blue-viewpoints. *Oral Surg Oral Med Oral Pathol* 1999; **87**: 526–527.
- EPSTEIN JB, OAKLEY C, MILLNER A, EMERTON S, VAN DER MEIJ E, LE N. The utility of toluidine blue application as a diagnostic aid in patients previously treated for upper oropharyngeal carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997; **83**: 537–547.
- HERLIN P, MARNAY J, JACOB JH, OLLIVIER JM, MANDARD AM. A study of the mechanism of the toluidine blue dye test. *Endoscopy* 1983; **15**: 4–7.
- HOROWITZ AM, DRURY TF, GOODMAN HS, YELLOWITZ JA. Oral pharyngeal cancer prevention and early detection: dentists' opinions and practices. *J Am Dent Assoc* 2000; **131**: 453–462.
- JOHNSON NW, WARNAKULASURIYA S, SPEIGHT P, EPSTEIN J. Diagnosing oral cancer: can toluidine blue mouthwash help? In: JOHNSON NW, ed: *Oral Cancer*. London: FDI World Dental Press 1999: 24–28.
- KLUG C, NEUBERG J, GLASER C, SCHWARZ B, KERMER C, MILLESI W. Quality of life 2–10 years after combined treatment for advanced oral and oropharyngeal cancer. *Int J Oral Maxillofac Surg* 2002; **31**: 664–669.
- LONKY NM, MANN WJ, MASSAD LS, MUTCH DG, BLANCO JS, VASILEV SA *et al.* Ability of visual tests to predict underlying cervical neoplasia: colposcopy and speculoscopy. *J Reprod Med* 1995; **40**: 530–536.
- MANN W, LONKY N, MASSAD S, SCOTTI R, BLANCO J, VASILEV S. Papanicolaou smear screening augmented by a magnified chemiluminescent exam. *Int J Gynecol Obstet* 1993; **43**: 289–296.
- MANUEL S, RAGHAVAN SK, PANDEY M, SEBASTIAN P. Survival in patients under 45 years with squamous cell carcinoma of the oral tongue. *Int J Oral Maxillofac Surg* 2003; **32**: 167–173.
- MARTIN IC, KERAWALA CJ, REED M. The application of toluidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; **85**: 444–446.
- MASHBERG A. Tolonium (toluidine blue) rinse—a screening method for recognition of squamous carcinoma: continuing study of oral cancer. *J Am Med Assoc* 1981; **245**: 2408–2410.
- MASHBERG A. Final evaluation of toluidine chloride rinse for screening of high-risk subjects with asymptomatic squamous carcinoma. *J Am Dent Assoc* 1983; **106**: 319–323.
- MURDOCH-KINCH CA. Oral medicine: advances in diagnostic procedures. *J Calif Dent Assoc* 1999; **27**: 773–784.
- NIEBEL HH, CHOMET B. In vivo staining test for delineation of oral intraepithelial neoplastic change: preliminary report. *J Am Dent Assoc* 1964; **68**: 801–806.
- PARHAM GP. Comparison of cell collection and direct visualization cervical cancer screening adjuncts. *Am J Obstet Gynecol* 2003; **188**: s13–s19.
- PORTER SR, SCULLY C. Early detection of oral cancer in the practice. *Br Dent J* 1998; **185**: 72–73.
- ROSENBERG D, CRETIN S. Use of meta-analysis to evaluate toluidine chloride in oral cancer screening. *Oral Surg Oral Med Oral Pathol* 1989; **67**: 621–627.
- SABES WR, SINGER RE, KUHN T. Effectiveness of toluidine blue as an aid to biopsy in the diagnosis of DMBA induced hamster pouch dysplasia and carcinoma. *Cancer* 1972; **29**: 1584–1589.
- SHEDD DP, HUKILL PB, BAHN S, FERRARO RH. Further appraisal of in vivo staining properties of oral cancer. *Arch Surg* 1967; **95**: 16–22.
- SIGURDSON A. Toluidine blue staining as a malignancy test. *Acta Otolaryngol* 1973; **75**: 308.
- STRONG MS, VAUGHAN CW, INCZE JS. Toluidine blue in the management of carcinoma of the oral cavity. *Arch Otolaryngol* 1968; **87**: 101–105.
- THOMSON PJ. Field change and oral cancer: new evidence for widespread carcinogenesis? *Int J Oral Maxillofac Surg* 2002; **31**: 262–266.
- WEIJERS M, SNOW GB, BEZEMER DP, VAN DER WAL JE, VAN DER WAAL I. The status of the deep surgical margins in tongue and floor of mouth squamous cell carcinoma and the risk of local recurrence; an analysis of 68 patients. *Int J Oral Maxillofac Surg* 2004; **33**: 146–149.

Address:

S. Ram

Department of Oral Pathology
Oral Medicine & Periodontology

Faculty of Dentistry
University of Malaya
50603 Kuala Lumpur
Malaysia

Tel: +60 3 79674803

Fax: +60 3 79674531

E-mail: saravanaram@yahoo.com