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

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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% tolonium 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 tolonium 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 tolonium chloride was 100% and 70.3%, respectively; and specificity was 14.2% for Vizilite® and 25% for tolonium chloride. Their accuracy was 80.6% and 64.5%, respectively. Current findings suggest that chemiluminescence is a more reliable diagnostic tool than tolonium chloride in the detection of oral cancer and PMELs, and for follow-up of patients treated for the same.

Key words: chemiluminescence; Vizilite®; tolonium 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 tolonium 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 Weidemann in 1888. Chemiluminescent reactions occur in the gas, solid and liquid state. In its simplest form it can be represented as:

$$A + B \rightarrow [I]* \rightarrow \text{products} + \text{light}$$

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

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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 day[1].

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 spectrum[1]. 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 dye[16]. 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 disease[7]. 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 treat[19].

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 structures[19]. 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 carcinoma[24]. 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 changes[4]. 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, raspberry 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