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Efficacy of light based detection systems for early detection of oral cancer and oral precancerous lesion

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Abstract
Carcinoma in an early stage of development is hard to detect clinically because the lesion may not be palpable and color of the lesional tissue is not necessarily different from the color of the surrounding mucosa. In order to improve the efficacy of the diagnosis, techniques are being developed to complement clinical examination and to facilitate the identification of initial carcinomas.

Aims: To find out the efficacy of chemiluminescent illumination (ViziLite™) for the diagnosis in precancer and cancer patients and compare this result to toluidine blue staining.

Materials and Methods: This study was done in 3 groups. Each group consists of 10 cases. Group I consists of normal appearing mucosa. Group II and III consist of clinically diagnosed precancer and clinically suggestive of cancer respectively. Chemiluminescent illumination, toluidine blue supravital staining and biopsy were performed in all cases. SPSS version 10.05 was used to calculate positive and negative predictive values.

Results: In Group I, all 10 patients showed negative result to ViziLite™. 8 patients showed positivity and 2 patients showed negativity to ViziLite™ test in Group II. 9 patients were positive and one patient was negative for ViziLite™.

Conclusions: Chemiluminescent illumination test was sensitive for precancerous and cancerous lesions, which presented as keratotic lesions and red-white lesions. It showed negative result to erosive lesions. Toluidine blue staining test was reliable in precancerous and cancerous lesions, which present as erosive and red-white lesions. It showed negative result to keratotic lesions. These results indicate that chemiluminescent illumination test is relatively reliable and accurate than toluidine blue staining test and useful chair side diagnostic test.

Keywords: Chemiluminescent illumination, oral exfoliative cytology, toluidine blue

Introduction
Oral malignancies are one of the most common cancers around the world and ranks sixth to eighth among cancers in various studies. These cancers are major economic and clinical burden for the health care around the world [1]. In India, oral cancer represents a major health problem accounting for upto 40 % of all cancers, and is most common cancer in males and third most common cancer in females. It often arises from Oral potential malignant disorders (OPMDs) such as erythroplakia, leukoplakia and oral Lichen planus [2]. Leukoplakia is the most common OPMD and its worldwide prevalence is approximately 2.6% [3]. Risk factors for oral cancer are well established and include tobacco and alcohol use [4]. Despite the established risk factors and advances in treatment, the 5-year survival for oral squamous cell carcinoma (OSCC) associated with tobacco and alcohol use has remained consistently poor for the last forty years [5]. Prognosis is further complicated by the high rate of second primary tumours in these patients, which is thought to be the result of 'field cancerisation' in the upper aerodigestive tract [6].

Early detection of neoplastic changes in the oral cavity is the best method to improve patient survival rates [7].

The current method of oral cancer diagnosis, visual examination of the oral cavity, relies heavily on clinical expertise in recognizing early neoplastic changes. However, discerning premalignant and early malignant lesions from common benign inflammatory conditions by visual examination is difficult, even for experienced practitioners [8]. Many techniques to date have been reviewed so far e.g. vital staining procedure (Toulidine Blue and Lugols iodine), Brush Biopsy (Oral CDx Brush), micronuclei anlaysis, DNA ploidy but have certain limitations [2]. Light-based techniques, including chemiluminescence and auto fluorescent imaging, work on the assumption that neoplastic and pre-neoplastic tissues that have undergone abnormal metabolic or structural changes have different absorbance and reflectance properties when exposed to specific wavelengths of light. The present study was done to compare the usefulness and validity of ViziLite™, toluidine blue *in vivo* application and oral exfoliative cytology with gold standard of biopsy.

Materials and Methods

This study was done in 3 groups. Each group consists of 10 cases. Group I consists of normal appearing mucosa. Group II and III consist of clinically diagnosed pre-cancer and clinically suggestive of cancer respectively. Chemiluminescent illumination, toluidine blue supravital staining, oral exfoliative cytology and biopsy were performed in all cases. A detailed case history was recorded. The patient consent was obtained. Chemiluminescent illumination (ViziLite™). The patients were instructed to rinse their mouth with the ViziLite™ rinse recommended (1% acetic acid solution). They were asked to swish the rinse all over the mouth for one minute and expectorate the contents. The examination room was dimmed to minimize ambient light. The ViziLite™ capsule was activated and assembled with the ViziLite™ retractor. The oral cavity was re-examined using the illumination from the ViziLite™ assembly. The observations were recorded accordingly and duly photographed. The ViziLite™ device was discarded. The presence of "acetowhite" lesion after one minute rinse with 1% acetic acid solution was considered as a "positive" test. The absence of such findings was considered as a "negative" test.

Toluidine blue

The toluidine blue technique was performed after the ViziLite™ procedure. The intraoral lesion was cleaned with 1% acetic acid. A cotton applicator tip was soaked with toluidine blue and applied over the lesion for 30 seconds. 1% acetic acid on a cotton applicator tip was used for 30 seconds to remove excess stain from the lesion. The observations were recorded in detail and lesion was photographed. Lesions that exhibited dark blue (or) stippled staining were considered as a "positive" test, while those that stained faintly (or) not at all were considered as "negative" test.

All the patients were subjected to subsequent biopsy. The areas as indicated by the positivity of ViziLite™ and toluidine blue were the preferable sites chosen for the biopsy. If the areas indicated by ViziLite ™ and toluidine blue were not same, biopsy was performed from each site indicated by their positivity.

Results

Data analysis and data base management was done using SPSS version 10.05. Descriptive analysis, sensitivity and specificity were calculated. Positive and negative predictive values were also calculated.

Group I

All 10 patients showed negative result to ViziLite™ and toluidine blue staining test. All 10 cases exhibit normal epithelium in histopathology [Table 1].

Group II

8 patients showed positivity and 2 patients showed negativity to ViziLite™ test. These 2 patients exhibited mild dysplasia6 patients showed positivity and 4 patients showed negativity to toluidine blue staining test. Of the 4 patients, 3 had mild dysplasia and one case showed moderate dysplasia in histopathology [Table 2].

Group III

9 patients were positive for ViziLite™ test and one patient was negative for ViziLite™ test. The negative case was diagnosed as moderately differentiated squamous cell carcinoma. All 10 cases showed positive result when subjected to toluidine blue staining [Table 3].

Table 1: Comparison of ViziLite™ and toluidine blue positivity in normal epithelium.

Histopathology	ViziLite™ positivity	Toluidine blue positivity
Normal epithelium (*n*=10)	00	00

Table 2: Comparison of ViziLite™ and toluidine blue positivity in precancer.

Histopathology	ViziLite™ positivity	Toluidine blue positivity
Hyperkeratosis (*n*=1)	1	1
Mild dysplasia (*n*=5)	3	2
Moderate dysplasia (*n*=3)	3	2
Severe dysplasia (*n*=1)	1	1

Table 3: Comparison of ViziLite™ and toluidine blue positivity in oral cancer

Histopathology	ViziLite™ positivity	Toluidine blue positivity
Carcinoma *in situ* (*n*=1)	1	0
Well differentiated (*n*=3)	2	1
Moderately differentiated (*n*=5)	5	0
Poorly differentiated (*n*=1)	1	0

Discussion

The early diagnosis and management of carcinoma of the oral cavity is essential and it continues to present a great challenge. Carcinoma in an early stage of development is

hard to detect clinically because the lesion may not be palpable and color of the lesional tissue is not necessarily different from the color of the surrounding mucosa [8]. In order to improve the efficacy of the diagnosis, techniques are being developed to complement clinical examination and to facilitate the identification of initial carcinomas [9]. A non-toxic chemiluminescent light source has been recently used to supplement clinical examination of precancer and cancer lesions. Chemiluminescent (ViziLite^{TM}) was first used by Huber *et al.* [3] to study mucosal abnormalities in populations at increased risk for oral cancer.

In 10 precancer patients, 4 patients had keratotic lesions, 4 had red-white lesions and 2 had erosive lesions clinically. Chemiluminescent light positivity observed in 8 patients, which presented clinically as keratotic and red-white lesions. On histopathological examination, 3 patients showed mild dysplasia, 3 patients showed moderate dysplasia and one showed severe dysplasia. But, one patient, who had a positive chemiluminescence showed hyperkeratosis without dysplastic changes on histopathology and this case was considered as false positive.

In 10 cancer patients, 6 patients presented with nonhealing ulcerative lesions clinically surrounded by white keratotic borders, 3 had red-white lesions and one patient had erosive lesions. When these patients were subjected to chemiluminescent light, 9 showed positive result. Of these 9 cases, 3 patients had well differentiated OSCC, 4 patients had moderately differentiated OSCC and one patient had poorly differentiated OSCC. But, one patient showed carcinoma *in situ* histopathologically. We considered the result of this case as a false positive.

Most probably the site subjected to biopsy in this lesion may not have the representative area to exhibit features of well differentiated OSCC histopathologically. To confirm this, we wanted to repeat the biopsy but the patient was not available for a follow-up. This may be extended to state that chemiluminescent light may be unable to distinguish epithelial dysplasia confined to the basement membrane from that of a well differentiated infiltrating carcinoma.

One patient with a clinically erosive lesion showed negative result to chemiluminescent light. Histopathologically, this patient showed well differentiated OSCC. This case was considered as "negative". From this, we observed that an erosive lesion when subjected to chemiluminescent light will elicit a false negative result.

Vahidy *et al.*, [10] Epstein *et al.*, [11] Warnakulasuriya *et al.* [12] have evaluated the efficiency of *in vivo* staining with toluidine blue in the detection of dysplasias and malignant lesions. Toluidine blue is an acidophilic dye of the thiazine group that selectively stains acetic tissue components such as DNA and RNA. Its use *in vivo* is based on the fact that dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissues. In addition, malignant epithelium may contain intracellular canals that are wider than in normal epithelium and this factor that could enhance the penetration of the dye [13]. Shedd *et al.* [14] stated that non dysplastic epithelium fails to retain toluidine blue.

Presently, we evaluated two diagnostic procedures that have been used as an aid in the diagnosis of oral cancer other than biopsy. Chemiluminescent illumination test was sensitive for precancerous and cancerous lesions, which presented as keratotic lesions and red-white lesions. It showed negative result to erosive lesions. Toluidine blue staining test was reliable in precancerous and cancerous lesions, which present as erosive and red-white lesions. It showed negative result to keratotic lesions. Sensitivity of chemiluminescent light (83.3%) was greater than that of toluidine blue (77.8%) and its positive predictive value (88.2%) and negative predictive value (76.9%) were also high compared to toluidine blue. These results indicate that chemiluminescent illumination test is relatively reliable and accurate than toluidine blue staining test and useful chair side diagnostic test.

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