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Study of Precancerous Lesions and Conditions by Clinical Examination, Chemiluminescence, and Toluidine Blue as Early Detection Tool- Retrospective Study.

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ABSTRACT

Background: Oral potentially malignant disorders constitute one of the major oral health problems in India. Oral cavity cancer accounts for approximately 4% of all malignancies and is a significant worldwide health problem. Therefore, 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). The present study was done to compare the usefulness and validity of clinical examination, chemiluminescent light kit or ViziLite and 1% toluidine blue in assessing the precancer. Aims and Objectives: To detect epithelial dysplastic changes using clinical examination, chemiluminescene (commercially available as ViziLite) and toluidine blue staining in Potentially Malignant Epithelial Lesions , Oral Squamous Cell Carcinoma patients and on clinically normal appearing oral mucosa of high risk (with habits) patients and compare the results obtained with histopathological examination. **Methods**: A total of 100 patients- 45 patients with PMELs, specifically oral leukoplakia, 15 patients with clinically diagnosed OSCC and 40 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 91.32% and 80.5% 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 84.66% and 72.7% respectively. **Conclusion**: ViziLite was relatively reliable in screening PMELs compared to toluidine blue, and was a useful early diagnostic tool.

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

INTRODUCTION

Oral potentially malignant disorders (OPMDs) include a variety of lesions and conditions characterized by an increased risk for malignant transformation. Oral cancer is a leading cause of morbidity and mortality.[1] It is the tenth most common cancer in the world and third most in India with a marked geographic difference in occurrence and hence remains a serious oral health problem worldwide.[1] The highest rates in the world for oral cancer are found in France, the Indian subcontinent, Brazil and Central/Eastern Europe.[2,3] The common occurrence among Indian population is attributed to the well-established association of oral cancer with betel quid chewing.[3] It has one of the lowest survival rates of 30%–80%, within a 5-year period.[2-4]

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Oral Squamous Cell Carcinoma (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.[3,4] The most commonly occurring oral precancers in Indian population include oral leukoplakia and oral submucous fibrosis(OSMF),out of which 8–10% eventually lead to malignancy.[5] The WHO reported oral cancer as having one of the highest mortality ratios among all malignancies, due to delayed diagnosis and the surgical treatment causing facial disfigurement, impaired speech and malnutrition.[2,6] The absence of a reliable method for early diagnosis of oral cancer is responsible for the delay in diagnosis and thus poor prognosis. It is therefore important to detect these lesions early to improve the prognosis with the help of better screening by use of various minimally invasive, diagnostic visualization aids such as toluidine blue, ViziLite. Hence the study was done to detect epithelial dysplastic changes using clnical

examination, chemiluminescene (commercially available as ViziLite) and toluidine blue staining in PMELs , OSCC patients and on clinically normal appearing oral mucosa of high risk (with habits) patients and compare the results obtained with histopathological examination.

MATERIALS AND METHODS

A total of 100 patients were selected from the out-patient department of Jawaharlal Nehru Institute of Medical Science, Porompat, Imphal, and study was conducted for a period of a year (2017-2018). 45 patients with PMELs, specifically oral leukoplakia. 15 patients with clinically diagnosed OSCC. 40 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. The ViziLite kit was used which 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 containing hydrogen peroxide. [Figure 1] Normal mucosa gave 'blue hue', which was considered negative for the test. 1% Toluidine blue solution was composed of tolonium 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.Area with no retention was considered negative for the test. Biopsy and histopathological analysis of the tissues were performed. The paraffin- embedded specimens were cut into 3–4 µm thick sections and stained with hematoxylin- eosin. The tissues were analyzed and were classified as negative (acanthosis, inflammatory lesions), positive which included dysplasia (subdivided into mild dysplasia, moderate dysplasia, severe dysplasia and carcinoma in situ), oral lichen planus, oral submucous fibrosis, proliferative verrucous leukoplakia and invasive carcinoma squamous cell carcinoma (SCC) and verrucous carcinoma. The definition of invasive carcinoma was based on the detection of infiltrative growth patterns for individual malignant cells or glands. Results from biopsy were considered as gold standard of diagnosis. Histopathological diagnosis of hyperkeratosis without dysplasia was considered a negative result and with dysplasia was considered a positive result 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. The data obtained were statistically analyzed. Sensitivity, specificity for the chemiluminescent technique and toluidine blue were calculated using the UK Centre for Evidence- Based Medicine online calculator for diagnostic test. Sensitivity, specificity were calculated using the following formulae:

Sensitivity = (true positives/true positive + false negative) ×100

Specificity = (true negatives/true negative + false positive) ×100.

RESULTS

The data collected was tabulated and subjected to statistical analysis. The tests were done for statistical analysis was Chi-square test and Kappa analysis. The results of ViziLite examination and toluidine blue are tabulated in [Table 1] P-value was found to be 0.006 and measure of Kappa analysis was 0.218. Clinically, leukoplakia was the most common finding with 61 cases (61.0%) either alone or in combination with other oral potentially malignant disorders, followed by oral submucous fibrosis (OSMF) followed by oral lichen planus, tobacco pouch keratosis, verrucous lesions and oral carcinoma. Chemiluminescence diagnosed 39 dysplasia, 09 carcinomas in situ, 05 cases of SCC and 05 cases of verrucous carcinoma to be positive giving true- positive results. Toluidine blue diagnosed 19 dysplasia, 05 carcinomas in situ, 09 SCCs, 06 verrucous carcinomas and 02 proliferative verrucous leukoplakia cases to be positive giving true- positive results. Few false positive and few false negative were also seen. Toluidine blue provided positive findings in 50 (50%) cases whereas chemiluminescence provided positive findings in 60 (75.0%) cases. (P = 0.006)

Histopathologically, dysplasia was the most common entity (in different grades) followed by OSMF. The diagnostic efficacy of both the tools was measured in terms of sensitivity, specificity. Sensitivity and specificity of ViziLite were calculated to be 91.32% and 80.5% respectively. Sensitivity and specificity of toluidine blue were calculated to be 84.66% and 72.7% respectively. The toluidine blue test was found to be moderately sensitive owing a specificity of 85.33%. The Vizilite was found to be highly sensitive with a sensitivity of 92.5%; however, the test has limited specificity of 52%. Our study also attempted to rule out whether ViziLite could distinguish lesions in clinically normal appearing mucosa without doing invasive biopsy procedure. 2 patient with habits, but no clinically visible lesion revealed positive test for ViziLite and toluidine blue in the right commissure of lip. The area was biopsied and revealed dysplasia. 1 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 were not willing for biopsy. 5 cases showed toluidine blue positivity inspite of normally appearing oral mucosa but were negative for ViziLite. [Figure 2 & 3]

Table 1: Correlation between toluidine blue and chemiluminescence positivity and histopathological findings

Histopthological findings	Total =100	Toulidine blue stain findings		Chemiluminescence findings	
		Negative	Positive	Negative	Positive
Carcinoma in situ	08	03	05	03	09
Mild Dysplasia	12	08	04	04	12
Moderate Dysplasia	23	09	14	00	22
Severe Dysplasia	05	04	01	00	05
Acanthosis	09	05	04	03	09
OSMF (Without dysplasia)	15	12	03	07	05
Lichen Planus	09	07	02	03	06
Squamous cell carcinoma	11	02	09	00	03
Proliferative verrucous leukoplakia	02	00	02	05	00
Verrucous carcinoma	06	00	06	00	04

κ=0.218; P=0.006.

Figure 1: showing vizlite kit or chemiluminescent illumination kit

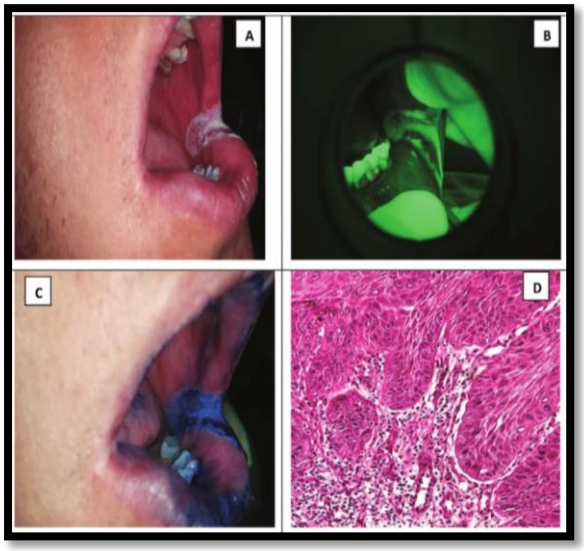

Figure 2: Showing oral squamous cell carcinoma clinically presenting in the form of speckled leukoplakia (b) chemiluminescence examination (c) Toluidine blue examination (d) Histopathology .

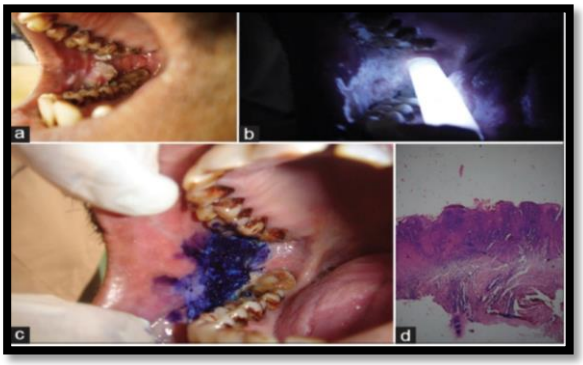

Figure 3: Verrucous carcinoma (a) clinical examination (b) chemiluminescence examination (c) Toluidine blue examination (d) Histopathology

DISCUSSION

As the incidence and death rate because of cancer have shown a sharp acceleration since the last two decades, more intense efforts are required to fight against this life-threatening disease.[6,7] In this study we evaluate the adjunctive utility of a chemiluminescent examination and application of a toluidine blue stain for detecting serious pathology associated with dysplasia when compared with the traditional clinical examination of the oral cavity. Toluidine blue is a cationic metachromatic dye that stains deoxyribonucleic acid and/or may be retained in intracellular spaces of dysplastic epithelium and clinically may appear as royal blue areas.[2] Theoretically, dysplastic and malignant cells have higher nucleic acid content than normal, and thus, staining of suspicious lesions with this dye can aid recognition of mucosal changes.[3-5] It is one of the most accepted screening tools used since a long time and has even been suggested as an alternative to frozen sections in developing countries. The usefulness and reliability of toluidine blue dye which binds to malignant or dysplastic tissues have been demonstrated in many studies.[2]

Chemiluminescence (Vizilite) is the other screening test used in the study which has been approved for use in the United States by the Food and Drug Administration since November 2001.

Normal epithelium will absorb chemiluminescent light and appear dark, whereas hyperkeratinized or dysplastic lesions appear white.[13-17] The difference in color could be related to altered epithelial thickness or to the higher density of nuclear content and mitochondrial matrix that preferentially reflects light in the pathological tissues.[18,21,22] Most of the studies have shown that chemiluminescence increases the brightness and margins of oral mucosal white lesions and thus assists in identification of mucosal lesions not considered under conventional visual examination.[11,14,19,20] Present study results showed sensitivity of ViziLite of 91.32%, which was close to the results of studies done by Ram and Siar 8.9and Camile S Farah et al,[10] where sensitivity was 100%. Our results were not in accordance with the studies of Ravi Mehrotra et al,[20] where sensitivity was 0%. The reason was because chemiluminescent (ViziLite) was unable to detect any true positive case out of three histopathologically positive cases while as specificity of ViziLite was found to be 80.5% which was not in accordance with other studies done by Ram and Siar,[8,9] where specificity was 14.2% and Camile S Farah,[10] 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 aceto white under chemiluminescent light.

Therefore, the results showed 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 defining the sharp borders between normal and abnormal oral mucosa. We also 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.[23-27] The reason may be accounted to false retention of stain in ulcerated and inflamed areas of the lesion. Present study also found that the chemiluminescence can detect early epithelial dysplastic changes in clinically normal appearing oral mucosa of high risk (with habits) patients. Changes may be molecular which might be occurring prior to cellular changes and which is impossible to detect even with histopathology. Whereas abnormal molecular changes take place even in normal appearing oral mucosa adjacent to or to the contra lateral side. It is assumed that the cause might be by the consumption of tobacco and intake of alcohol in these patients.

CONCLUSION

Chemiluminescence and toluidine blue are useful noninvasive methods for early detection of oral cancer but both cannot be compared with histopathology. Their adjunctive value is of great importance and should always be used as a chair-side investigation and for mass screening of oral cancer. Future studies with larger sample size on all types of precancerous population are needed.

REFERENCES

1. Park's Textbook of Preventive and Social Medicine. 21st ed. Goodreads. Available from: https://www.goodreads.com/work/
2. Abhilasha Shukla, Narendra Nath Singh, Sangeeta, Sulabh Kumar, Deepika Shukla, Anubhuti Sood et al. Chemiluminescence and toluidine blue in the detection of oral potentially malignant and malignant disorders. Journal of Oral and Maxillofacial Pathology ; 2018;22 (3)
3. Johnson N. Tobacco use and oral cancer: A global perspective. J Dent Educ 2001;65:328- 39.
4. Downer MC, Evans AW, Hughes Hallet CM, Jullien JA, Speight PM, Zakrzewska JM, et al. Evaluation of screening for oral cancer and precancer in a company headquarters. Community Dent Oral Epidemiol 1995;23:84- 8.
5. Narasannavar DA, Wantamutte DA. Prevalence of oral precancerous lesions and conditions among tobacco consumers in rural population around Belgaum. A community based cross sectional study. IOSR J Dent Med Sci 2014;13:31- 4.
6. Ambekar DM, Chaudhary BJ, Kulkarni VV. A study of prevalence of oral precancerous lesions in relation to tobacco habituation. Int J Med Clin Res 2014;5:282.
7. Petersen PE. Strengthening the prevention of oral cancer: The WHO perspective. Community Dent Oral Epidemiol 2005;33:397-9.
8. Bettendorf O, Piffkò J, Bànkfalvi A. Prognostic and predictive factors in oral squamous cell cancer: Important tools for planning individual therapy. Oral Oncol 2004;40:110-9.
9. Mark W Lingen, John R Kalmar, Theodore Karrison, Paul M Speight. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncology. 2008;44: 10–22.
10. Bouquot JE, Suarez P and Vigneswaran N. Oral Precancer and Early Cancer Detection in the Dental Office – Review of New Technologies. The Journal of Implant & Advanced Clinical Dentistry.2010;2:47-63.
11. Stefano Fedele. Diagnostic aids in the screening of oral cancer. Head & Neck Oncology. 2009;1-5
12. Scully C, Bagan JV, Hopper c and Epstein JB. Oral cancer: Current and future diagnostic techniques. Am J Dent.2008;21:199-209.
13. S Ram, C H Siar. Chemiluminescence as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions. Int J Oral Maxillofac Surg.2005;34:521-7.
14. Michaell A Huber, Samer A Bsoul and Geza T Terezhalmy. Acetic acid wash and chemiluminescent illumination as an adjunct to conventional oral soft tissue examination for the detection of dysplasia: A pilot study. Quintessence Int.2004;35:378-84.

15. Camile S Faraha, Michael J McCulloughb. A pilot case control study on the efficacy of acetic acid wash and chemiluminescent illumination (ViziLite™) in the visualisation of oral mucosal white lesions. Oral oncology.2007;48:820-82.
16. Kerr AR, Sirois DA, Epstein JB. Clinical evaluation of chemiluminescent lighting: an adjunct for oral mucosal examinations. J Clin Dent.2006;17:59-63.
17. 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; 43:289–96.
18. Rhys Carlson, Simon W. Lewis and Kieran F. Lim. Seeing the light: Using chemiluminescence to demonstrate chemical fundamentals. Chemeda: Aust. J. Chem. Ed., 10 April 2000.
19. Epstein JB, Gorsky M, Lonky S, Silverman S Jr, Epstein JD, Bride M. The efficacy of oral lumenoscopy (ViziLite) in visualizing oral mucosal lesions. Spec Care Dentist.2006; 26:171-4.
20. Esther S. Oh, Daniel M. Laskin. Efficacy of the ViziLite System in the Identification of Oral Lesions.Journal of Oral & Maxillofacial Surgery.2007;65:424-6.
21. Mehrotra, Singh, Thomas, Nair, Pandya, Nigam, et al. A Cross-Sectional Study Evaluating Chemiluminescence and Autofluorescence in the Detection of Clinically Innocuous Precancerous and Cancerous Oral Lesions; J Am Dent Assoc.2010;141:151-6.
22. Rebekah Drezek, Martial Guillaud, Thomas Collier, Iouri Boiko, Anais Malpica, Calum Macaulay, et al. Light scattering from cervical cells throughout neoplastic progression: influence of nuclear morphology, DNA content, and chromatin texture. Journal of Biomedical Optics.2003;8:7–16.
23. Vahidy NA, Zaidi SHM and Jafarey NA. Toluidine blue test for detection of carcinoma of the oral cavity: an evaluation. Journal of Surgical Oncology.1972:434-8.
24. Paloma Cancela-Rodríguez, Rocío Cerero-Lapiedra, Germán Esparza-Gómez, Silvia Llamas-Martínez, Saman Warnakulasuriya. The use of toluidine blue in the detection of pre-malignant and malignant oral lesions. Journal of Oral Pathology & Medicine.2011;40:300–4.
25. Imtiaz Ather Siddiqui, M Umer Farooq, Riaz Ahmed Siddiqui, SM Tariq Rafi. Role of Toluidine Blue in Early Detection of Oral Cancer. Pakistan Journal of Medical Sciences.2006;22:184-7.
26. JB Epstein, S Silverman Jr, JD Epstein, SA Lonky and MA Bride. Analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and toluidine blue. Oral Oncology.2008;44:538-44.
27. E Allegra, N Lombardo, L Puzzo1, A Garozzo. The usefulness of toluidine staining as a diagnostic tool for precancerous and cancerous oropharyngeal and oral cavity lesions. Acta Otorhinolaryngologica Italica.2009;29:187-90.
28. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. Int J Oral Sci 2013;5:59- 65.

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