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Non -invasive diagnostic tools in early detection of oral epithelial dysplasia

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Abstract

Objective: The incidence of oral cancer worldwide varies 2-18% and in India 0.1 to 13.5%. Early evaluation of oral precancerous lesions can have dramatic effect on oral cancer mortality rate. Among the premalignant stage, leukoplakia is the most commonly encountered clinical lesion and epithelial dysplasia is most important predictive factor. The objective of this paper is to compare the efficacy of exfoliative cytology, toluidine blue and chemiluminescent illumination (VIZILITE) to detect early dysplastic changes in leukoplakia. Study design: Study involved 50 patients of leukoplakia (homogeneous, speckled) in the age group of 20-72 years including males and females in the ratio of 7:3. All the diagnostic procedures were carried out on each patient followed by biopsy and data was subjected to statistical analysis.

Results: Comparing the results of exfoliative cytology with toluidine blue in diagnosing dysplasia in leukoplakia, it showed 50% sensitivity and 83.3 % specificity. In comparison to chemiluminescent light examination, cytology showed 42.9% sensitivity and 79.3% specificity. Chemiluminescent light examination showed 60% sensitivity and 70% specificity compared to toluidine blue.

Conclusions: Overall accuracy of exfoliative cytology was less than toluidine blue, whereas latter showed superior but comparable results to chemiluminescent illumination in detecting dysplasia. However role of chemiluminescent illumination should be further investigated in demarcating dysplatic lesions.

Key Words: *Oral cancer, leukoplakia, dysplasia, cytology, toluidine blue, chemiluminescence.*

Introduction

It has been well recognized since the beginning of this century that oral cancer is one of the commonest cancer in India (1). Oral cancer is major cause of death and disease throughout the world. The incidence of oral cancer worldwide varies 2-18% and in India 0.1 to 13.5%. Habits like smoking and chewing tobacco products is responsible for high incidence of oral cancer (2). Early evaluation of oral precancerous lesions can have dramatic effect on oral cancer mortality rate. The five-year survival rate for patients with early, localized disease approximately 80%, for those with distant metastasis, it is 19% (3). Clinical observations indicate that in a proportion of cases of squamous cell carcinoma are preceded by or co-exist with other distinct oral epithelial lesions. These precursor lesions are the ones, which have been designated as premalignant. Among the premalignant stage, leukoplakia is the most commonly encountered clinical lesion. Furthermore they often appear innocuous, since the classical clinical characteristics associated with advanced oral cancer including ulceration, induration, elevation, bleeding and cervical lymphdenopathy usually are absent in early stage lesions (4).

Premalignant potential of oral leukoplakia may be related to etiological, topographical, clinical or histological characteristics (5). Leukoplakia has varied appearance and although certain clinical features may indicate the lesion that have greater risk of becoming malignant, leukoplakia that histologically display severe dysplasia. The significance of the leukoplakia lesion, the most common precursor of oral cancer (85% of all precancerous lesions are leukoplakia) also has important prognostic implications (3). It has been proposed that the characteristics of cells from cancer lesions could provide the basis for diagnosis. Since then the classical studies of papanicolaou and other authors have demonstrated that cytology is both useful and reliable in early diagnosis of cancer (6). Use of 1% toluidine blue to see intra epithelial neoplastic changes had also been mentioned due to the property of toluidine blue to stain hyperchromatic nuclei (7). Huber et al. (8) in his pilot study found the usefulness of diffuse, low level blue white chemiluminescent light to detect early dysplastic changes in oral cavity. Scalpel biopsy is an invasive procedure associated with potential morbidity. Thus, many oral lesions undergo biopsy only when they display either symptoms or clinical features typical of malignancy, while many innocuous appearing early state oral cancerous lesions are merely observed clinically and left undiagnosed. Thus, in light of the need for more precise methods of identifying oral cancers in its early stages an attempt is made in this study to estimate the efficacy of various, non-invasive diagnostic methods to detect early dysplastic changes in leukoplakia.

Materials and methods

Fifty patients visiting to Department of Oral Medicine and Radiology, Government Dental College and research institute Bangalore were included in the study. The study was conducted according to ethical guidelines approved by Rajiv Gandhi University of Health Sciences Bangalore. Those patients selected for this study were in the age group of 20-72 years, 35 were male patients, and 15 were female patients. Only inclusion criterion for this study was clinical diagnosis of leukoplakia (homogenous and speckled type). A detailed case history of patient with emphasis on their habits and thorough clinical examination was recorded. Patients selected for the study were explained in detail about the lesion affecting and the diagnostic procedure. A formal informed written consent was obtained from all of them. Intraoral examination was conducted by trained specialists in oral medicine using dental mirrors and gauze and manual palpation of lesion.

Fig 1. *Preparation of pap smear*

For exfoliative cytology lesion was scrapped with metal spatula and material is spread immediately on the slide. Another slide is used to draw material across first slide. As soon as material is placed on a slide, it is fixed with alcohol spray, because slightest air-drying will cause distortion of the cells and make interpretation impossible. (Fig.1). Smears were stained by standard Papanicolaou's technique and studied for changes of character of cell population and individual cells. After examining the smear results were classified as described by Papanicolaou (9). Oral examinations for toluidine blue was done by cleaning with 10% H_2O_2 to remove the saliva, food, or tissue remains followed by 1% acetic acid wash. 1% solution of toluidine blue was applied with cotton tip for 30 seconds. (Fig.2). Excess stain was eliminated by applying 1% acetic acid for 30 seconds. Lesion was examined to see the size of retained stained areas. Interpretation of the stain was done as mentioned by Mashberg(10). The intraoral examination was repeated with chemiluminescent illumination

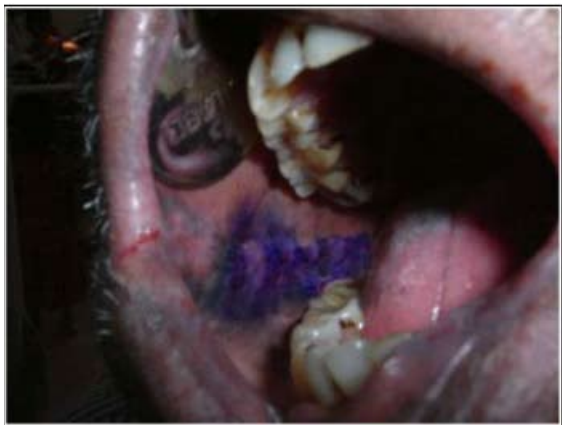

Fig 2. *Staining the lesion with toludine blue*

i.e. Vizilite, (Zila pharmaceuticals, Phoenix, Arizona, U.S.A.) Patients were instructed to rinse with 1% acetic acid solution for one minute and chemiluminescent capsule was activated, and assembled. The flexible outer capsule was bent and the brittle inner vial was broken. Then the capsule was shaken to mix contents. The chemiluminescent capsule was inserted into open piece of retractor, and the two piece retractor unit was assembled. Ambient lights in the room were then dimmed and oral soft tissue examination (visual inspection) was carried out under chemiluminescent illumination to detect abnormal epithelial changes. Normal epithelium gave blue hue and altered epithelium appeared "acetowhite" under chemiluminescent light. (Fig.3). All the findings of exfoliative cytology, 1% toluidine blue and vizilite were recorded for each lesion. After performing all the procedures punch biopsy was performed under local anesthesia for definite histopthological diagnosis. Fixed tissue was stained with haematoxylin and eosin and interpreted by independent oral pathologist who was not part of this study. Sensitivity, specificity, positive and negative predictive value for each technique was calculated and data was subjected to further statistical analysis.

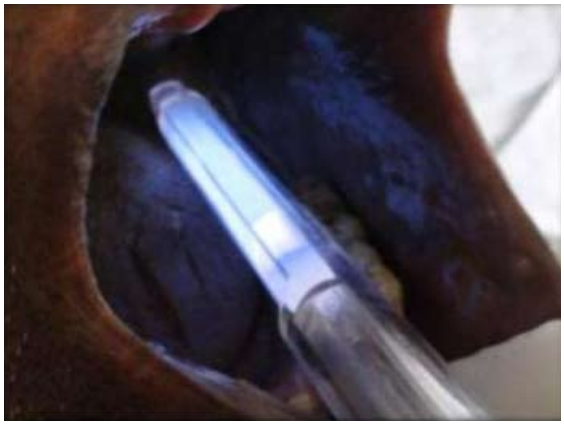

Fig 3. *Lesion under chemiluminescent illumination depicting acetowhite area*

Results

This study involved 50 patients of leukoplakia (homogeneous, speckled) with mean age of 44.34 ± 10.78 (mean ± standard deviation) years. Maximum incidence was found to be in the age group of 40-49 years. Among the study population 48% were smokers and 52% were tobacco chewing. Buccal mucosa was the most common site, 35 cases (70%) involved in our study followed by commissures 9 cases (18%), floor of mouth 3 cases (6%) and tongue 3 cases (6%). The clinical type most commonly found in the study was homogeneous type 37 cases (74%) and followed by speckled 13 cases (26%). Exfoliative cytology detected overall 11(47.8%) true positive cases and four cases were false positive. Twelve cases were false negative, and twenty three true negative. Overall sensitivity of exfoliative cytology in detecting dysplasia was 47.8% whereas specificity was 85.2% with positive predictive value (PPV) as 73.3% and negative predictive value (NPV) 65.7 % (Table 1).

		Biopsy		Total
		Positive	Negative	
Exfoliative Cytology	Positive	11 (47.8%)	4 (14.8%)	15 (30%)
	Negative	12 (52.2%)	23 (85.2%)	35 (70%)
Total		23	27	50
PPV		-	-	73.3%
NPV		-	-	65.7%

PPV- POSITIVE PREDICTIVE VALUE NPV- NEGATIVE PREDICTIVE VALU

Table 1. *Sensitivity and specificity of exfoliative cytology*

Toluidine blue examination detected dysplastic changes in 13 cases, out of 23 biopsy proven dysplasias. Twenty cases were true negatives, seven false positive and ten were false negative. Overall sensitivity of toluidine blue was 56.5% whereas specificity was 74.1% having PPV as 65% and NPV 66.7 %.(Table 2).

		Biopsy		Total
		Positive	Negative	
Toluidine Blue	Positive	13 (56.5%)	7 (25.9%)	20 (40%)
	Negative	10 (43.5%)	20 (74.1%)	30 (60%)
Total		23	27	50
PPV		-	-	65%
NPV		-	-	66.7%

PPV- POSITIVE PREDICTIVE VALUE NPV- NEGATIVE PREDICTIVE VAL

Table 2. *Sensitivity and specificity of toluidine blue*

Out of 23 biopsy proven dysplastic cases, 16 were found dysplastic (acetowhite) during chemiluminescent examination. This showed 7 cases as false negative, 22 cases as true negative and five cases were false positive. Overall sensitivity of chemiluminescent illumination was 69.6% and specificity was 81.5% with PPV 76.2% and NPV 75.9 %.(Table 3). Comparing the results of exfoliative cytology with Toluidine blue in diagnosing dysplasia in leukoplakia, it showed 50% sensitivity and

83.3 % specificity. In comparison to chemiluminescent light examination, cytology showed 42.9% sensitivity and 79.3% specificity. Chemiluminescent light examination showed 60% sensitivity and 70% specificity compared to toluidine blue.

		Biopsy		Total
		Positive	Negative	
Chemiluminescent illumination	Positive	16 (69.6%)	5 (18.5%)	21 (42%)
	Negative	7 (30.4%)	22 (81.5%)	29 (58%)
Total		23	27	50
PPV		-	-	76.2%
NPV		-	-	75.9%

PPV- POSITIVE PREDICTIVE VALUE NPV- NEGATIVE PREDICTIVE VALUE

Table 3. *Sensitivity and specificity of chemiluminescent illumination (VIZILITE) acetowhite area*

Discussion

It is well known fact that cells obtained from surface of tumor, often can provide a clue to diagnosis. Greatest value of cytological examination lies in its ability to disclose the presence of intraepithelial non-invasive carcinoma when the clinical appearance is relatively innocent and cancer is not suspected. Biopsy unquestionably provides a reliable means of obtaining a diagnosis but suggestion of biopsy may cause considerable emotional upset in cancerophobic patients, whereas surreptious scrapping can go a long way towards conforming a diagnosis without necessarily alarming the patients (6). Low sensitivity of exfoliative cytology in detecting atypical or dysplastic cells can be attributed to few reasons. The first was apparent sparsity of atypical cells in the smears from leukoplakia or leukoplakia with early malignant change. Most of these lesions exhibited maximum nuclear abnormalities in the lower squamous layers, as the cell progresses upwards, the nuclear atypism becomes less and less, resulting in superficial strata of epithelium in which cells showed little or no atypism. Secondly, the viable epithelium is covered by thick scales of squamous cell ghosts with pyknotic or with no nuclei. False negative results can be attributed to high keratinization or sample of the cells not representing the pathologic process (11). Since toluidine blue is regarded as nuclear stain, selective dye uptake can be due to more nuclear acids present in dysplastic and malignant cells as compared to normal tissues . It has been demonstrated that interfacial and intercellular canals that are present in normal epithelium are also present between tumor cells. Due to haphazard arrangement of tumor cells it appears likely that intercellular cannaliculi are much larger than the normal epithelium, consequently allowing more intensive penetration of dye (12). High false negative results found with the toluidine blue in our study can be due to the fact that surface layer of keratin contains pyknotic or no nuclei. False positive results found in the study were 25.9%, which can be attributed to false interpretation or due to retention of dye in fissures in leukoplakia (13).

Use of chemiluminescent illumination to detect the cervical neoplasia after acetic acid wash has been mentioned in different studies (14, 15). It has been speculated that oral soft tissues exhibit features similar to the cervical epithelium following an acetic acid wash and visual inspection under chemiluminescent illumination (8) and it is non-toxic to biological tissues (16) can be useful method to detect early dysplastic changes in oral mucosa. Chemiluminescent illumination used in our study is produced by fracture of two chamber capsule system, whereby esters of oxalic acid are oxidized in the presence of aromatic compounds (tri- colored phosphors). Properties of chemiluminescent light, which make it useful for detection of dysplasia as: (a) Multichromatic low intensity light with peak intensity less than 60 lumens with three spectral output peaks between 430-580 nm which appears blue white to examiner. (b) Cylindrical shaped chemiluminescent capsules provide uniform sheets of light to all mucosal surfaces about its axis with no shadows. (c) It is theorized that low intensity multichromatic light differentially absorbed or reflected by tissues of different densities (nuclear-cytoplasmic ratios), accentuating reflective contrast (demarcation) between normal and pathologic epithelium. (d) Low level light output with minimum glare and photo enhanced chemiluminescence effect (17). Overall sensitivity of chemiluminescent illumination in our study was 69.6%, whereas in cases of speckled type of leukoplakia it was 81.8% with positive predictive value of 90%. Epstein et al. (18) reported enhanced lesion brightness or sharpness in 61.86% of the cases and found that 55% of lesions of concern (dysplastic lesions) more easily visualised with vizilite. Unfortunately, given the design of that study, true sensitivity and specificity could not be determined for the examinations. Lingen et al. (19) and Patton et al. (20) in their critical and systematic review on diagnostic aids for the detection of oral cancer mentioned that the main problematic issues associated with these studies are their mixed results. Overall, toluidine blue appears to be good at detecting carcinomas but positive only 50% lesions with dysplasia. Given the variability in study design and fact that all levels of disease are reported in some studies it is difficult to estimate the effectiveness of toluidine blue staining in identifying dysplastic lesions. Enhanced visualization associated with vizilite does not necessarirly mean that the enhancement is restricted to suspicious lesions only. Evidence that supports the use of chemiluminescence system to aid in the detection of oral premalignant lesions is currently quite sparse. The published studies suffer from numerous experimental design issues, especially the critical comparison to diagnostic gold standard (scalpel biopsy) in all cases.

In order to improve patient prognosis, early detection

and treatment of premalignant and malignant lesions is essential. Exfoliative cytology appears to be of limited success in cases of homogenous type of leukoplakia, whereas in speckled and ulcerative type of leukoplakia it is reasonably reliable method in diagnosing dysplasia in this research study. Toluidine blue application revealed superior results in detecting dysplasia as compared to cytology. Toluidine blue was highly reliable in cases of speckled and ulcerative types of leukoplakia. Results obtained with vizilite were better than cytology and slightly superior to Toluidine blue in detection of dysplasia. Regardless of results of this study cost effectiveness of toluidine blue application as diagnostic adjunct is much better than the vizilite, which is an expensive product with marginally improved results. Thus, clinically there is insufficient evidence to support the use vizilite as an adjunct to toluidine blue since it provides limited benefit for additional cost incurred. In an effort to enhance the usefulness of vizilite system, modified version (vizilite-plus) has been introduced by manufacturers which contains toluidine blue (Tblue) inside the kit to mark acetowhite areas with blue stain as a guide to the biopsy site once the light source is removed. In this study older version (vizilite) was used which do not contain Tblue inside the kit. Therefore, the time interval and sequence of toluidine blue application and vizilite did not affect the outcome of this study.

From the data collected from this study, it can be concluded that role of exfoliative cytology in detecting dysplasia in leukoplakia has certain limitations, whereas accuracy, sensitivity, predictive values of toluidine blue application is certainly superior to exfoliative cytology. Role of chemiluminescent examination following an acid wash in detecting dysplasia was slightly superior but comparable to toluidine blue. However, further studies are required to evaluate the full potential of vizilite as a diagnostic adjunct in demarcating dysplastic lesions.

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