INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

IJCMPR

Available Online at http://www.journalcmpr.com

RESEARCH ARTICLE

DIAGNOSTIC EFFICIENCY OF TOLUDINE BLUE IN DETERMINING PREMALIGNANT AND EARLY MALIGNANT LESIONS

Darshana Warke and Komal Khot

Department of Oral pathology and microbiology

ARTICLE INFO

Article History:

Received 8th August, 2016
Received in revised form 18th September, 2016 Accepted 24th October, 2016 Published online 28th November, 2016

Key words:

Toludine blue , Premalignant lesions , malignant lesions , epithelial dysplasias..

ABSTRACT

Oral cancer when caught at an early stage is often curable, inexpensive to treat and affords a better quality of life. With this aim various techniques have been developed to supplement clinical examination and improve the diagnosis of premalignant and early malignant lesions.

Aims and objective: The objective of this study is to determine the diagnostic efficiency of Toludine blue in oral premalignancies and malignancies and to analyse the reliability of in vivo staining with Toludine blue in the lesions at risk of malignancy and its correlation to epithelial dysplasias seen in histological sections.

Materials and methods: Study was conducted in the Department of Oral and Maxillofacial Pathology, Y.M.T Dental College, Navi Mumbai. The study group comprised of 25 subjects with clinically suspicious premalignant lesions and 25 subjects with clinically suspicious malignant lesions. Depending on the retention of dye, the biopsy site was determined. The biopsy specimen was sent for histological confirmation.

Results: Premalignant lesions and conditions take up Toludine blue stain in deeper intensities depending on the severity of dysplasia.

Conclusion: Toludine blue staining can be used as a supplement to aid in diagnosis of premalignant and malignant lesions.

INTRODUCTION

Oral cancer is often diagnosed when patients already have advanced disease and thus have poor prognosis. Dysplasia may be present in upto 16% of potentially malignant oral lesions (PMOL's) & 10% of suspected PMOL's may be malignant at time of diagnosis (Silverman *et al*., 1976; Pindborg *et al*., 1977). Malignant transformation has been reported in upto 43% of leukoplakia. Thus early diagnosis is of paramount importance (Pindborg *et al*., 1968). Although detection methods have improved by leaps & bounds, even today half of the afflicted patients remain undiagnosed. Of the several pre-surgical assessment aids vital dyes being simple & inexpensive have been widely applied in clinical practice and have been used extensively. Toludine blue, discovered during 1960s, is a basic metachromatic dye of thiazine group that shows affinity for the perinuclear cisternae of DNA and RNA (Herlin *et al*., 1983). Toludine blue selectively stains acidic tissue components, DNA and RNA, and as the dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissues, these can show a varied clinical picture when stained with toludine blue. (Allen *et al*., 1949) introduced the therapeutic use of tolonium chloride as an i/v anti-heparin agent. In 1960 Sherwin suggested to (Strong *et al*., 1968) the use of tolonium chloride for in-vivo staining of suspicious lesions of oral cavity that might stain tumor cells and normal mucosa or leukoplakia differentially. Reichart (1963) first reported the use of 1% tolonium chloride stain in delineation of neoplastic epithelium of the cervix. Its use in- vivo is based on the fact that dyslastic and anaplastic cells contain quantatively more nucleic acids and increased mitoses than normal surrounding epithelium (Vercellino *et al*., 1985). Another mechanism appears to be greater penetration and temporary retention of the dye in the intercellular spaces of rapidly dividing cells in-vivo RNA (Herlin *et al*., 1983).[1]

Aims and Objectives

To determine the diagnostic efficiency of toludine blue in oral premalignancies and malignancies and its correlation to epithelial dysplasias seen in histological sections. 25 clinically suspicious premalignant lesions and 25 clinically suspicious malignant lesions and conditions have been evaluated.

MATERIALS AND METHODS

This Study has been conducted in the department of Oral and Maxillofacial pathology, YMT Dental College, Navi Mumbai. All clinically suspicious lesions were stained with toludine blue and dyeretention was evaluated.

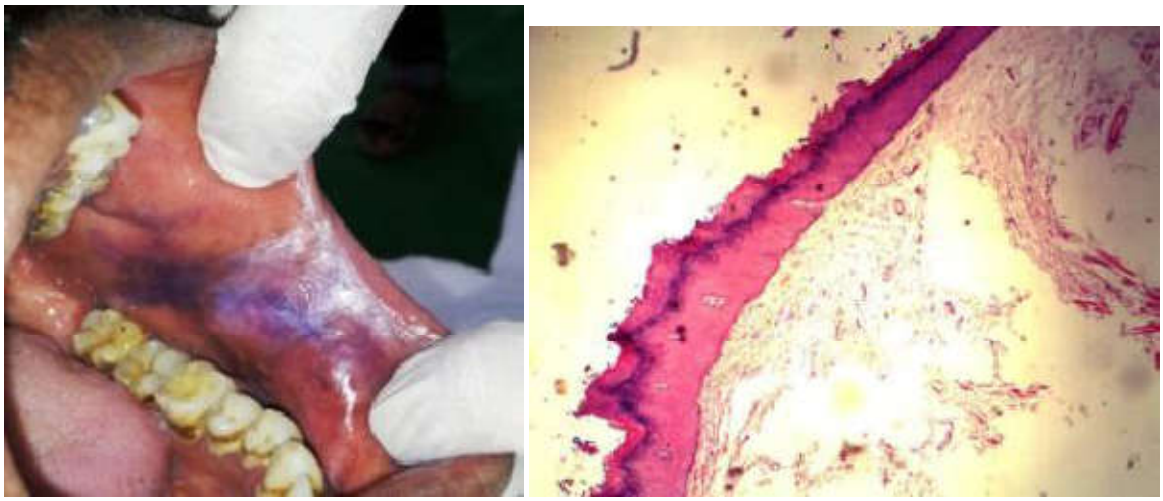

Fig. A Leukoplakia which has been stained mildly with toludine blue stain prior to taking biopsy. Histopathological picture showed hyperkeratosis on biopsy.

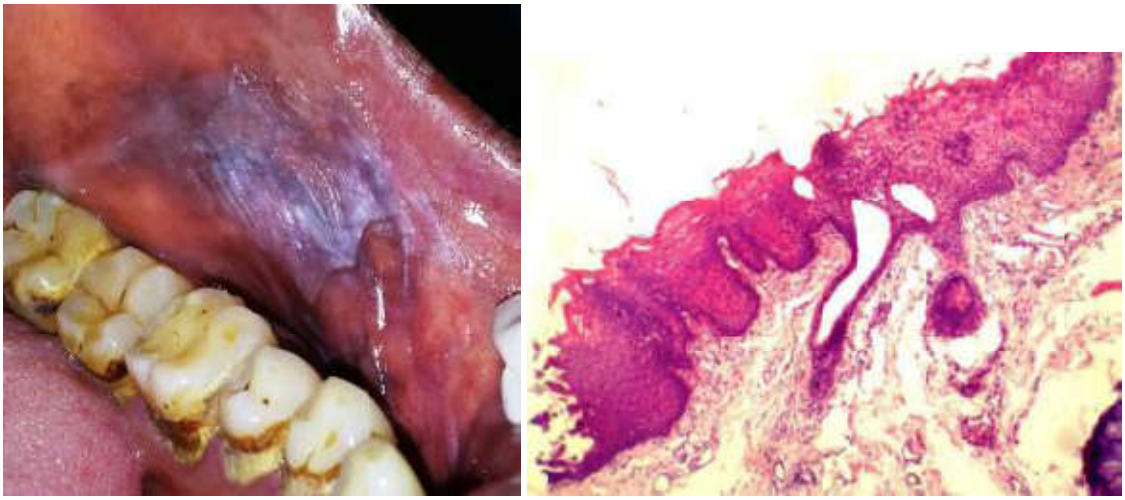

Fig B Leukoplakia stained moderately with Toludine blue stain prior to biopsy. Histopathology revealed mild to moderate dysplasia.

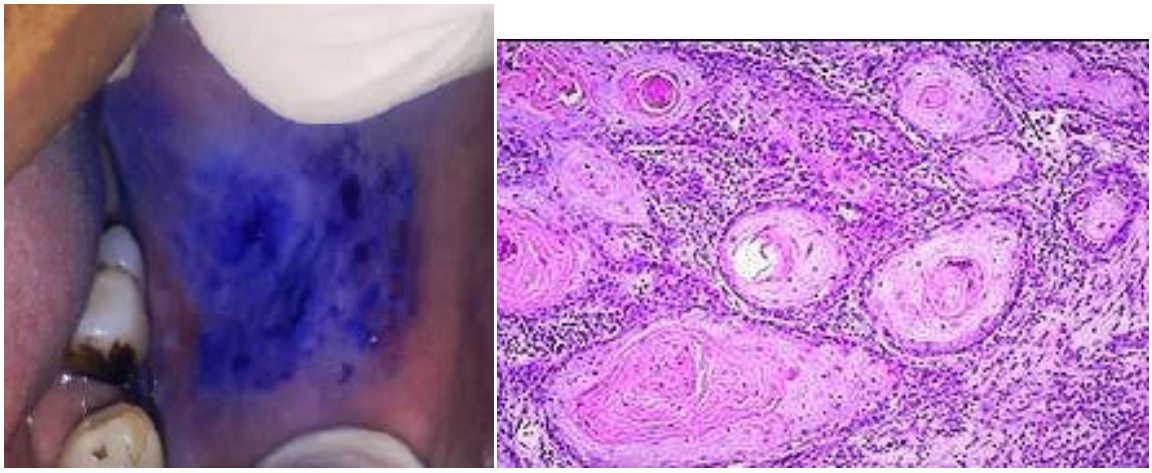

Fig C Leukoplakia stained deeply with Toludine blue stain prior to biopsy. Histopathology revealed severe dysplasia.

Of the 50 cases that were evaluated , 11 cases of OSMF , 10 cases of epithelial dysplasia , 25 cases of keratotic lesions and 1 case of erythroplakia and 3 cases of squamous cell carcinoma were observed. Lesions that were mildly stained with toludine blue showed hyperkeratosis and mild dysplasias on histopathology, lesions which were moderately stained with toludine blue showed moderate dysplasia on histopathology and lesions which were deeply stained by toludine blue showed malignant changes on histopathological examination.

In a few cases false positive results were noted .Of 50 cases evaluated 7 gave false positive results. Also the above study is subjective to examiners evaluation.

RESULTS

Premalignant lesions and conditions take up toludine blue stain in deeper intensities depending on the severity of dysplasia. Mild stain was taken up by hyperkeratotic lesions and mild dysplastic lesions, moderately stained sections

demonstrates moderate dysplasia , and severely stained lesions demonstrates either carcinoma in situ or squamous cell carcinoma.

CASES (n = 50)	Dysplasia	NO Dysplasia
Stain positive	35	8
Stain negative	1	6

DISCUSSION

Oral cancer is usually first diagnosed when it becomes symptomatic. By this stage approximately 2/3rd of the patients will have already developed advanced disease with regional metastasis (clinical or microscopic metastasis). The disease is life threatening, with high morbidity resulting from late treatment. However, if it is diagnosed at an early stage, oral cancer is often curable and inexpensive to treat.[7] In vivo staining has been used extensively in gynaecologic practice for the detection of malignant changes of cervix , and this technique has been applied in the oral setting for over 30 years by means of dyes like toludine blue. Toludine blue selectively stains acidic tissue components, DNA and RNA , and as the dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissues, can show a varied clinical picture when stained with toludine blue.[2] Hence it is important to study the diagnostic efficiency of toludine blue within detecting the biopsy sites and thus establishes an accurate diagnosis in oral premalignant and malignant lesions.

Present study gives us 14% of false positive results attributed to epithelial hyperplasia and hyperkeratotic lesion. Out of 50 cases, 35 cases that took up the stain elicited dysplasia.8 cases which took up positive stain showed no dysplastic features. One case stained negative for toludine blue but elicited dysplastic features on histopathological examination. The false negative result could be due to improper technique. 6 cases did not take up the stain and did not elicit dysplasia. Mild stain was taken up by hyperkeratotic lesions and mild dysplastic lesions which were 16 in number, moderately stained sections showed moderate dysplasia which were 15 cases , and severely stained conditions which were 4 cases showed either carcinoma in situ and squamous cell carcinoma.

Various studies have been carried out to study the diagnostic efficiency of toludine blue in determining epithelial dysplasias. 7 cases out of 50 elicited false positive results. Mashberg (1980) reported false negative of 75%, (Martin *et al*., 1998) gave 58%, (Onofre *et al* 2001) 6%, whereas (Zhang *et al*., 2005) reported 55%.As pointed out by (Epstein *et al*., 2003) the detection of low-grade (mild/moderate) oral dysplasia has been less consistent, with a significant portion of such lesions not staining with toluidine blue, (Zhang *et al*., 2005) failed to detect 77% of low-grade dysplasia, and upto 64% of the PMOL's were TBN. Blinded reliability on staining thus results in underdiagnosis, which is not justified since malignant transformation of 3-5% has been reported in mild and 3-15% for moderate dysplasia (Speight 2007). Toludine blue appears to stain only three to four cells deep and thus reflects changes in the epithelial layer alone. Invaded underlying tissue is not penetrated by the dye and likewise the extent of submucosal spread is difficult to appreciate Mashberg (1981). Epstein failed to elicit any difference between clinical examination and toludine application in the detection of dysplastic lesions (Epstein *et al*., 1997). (Kerawala *et al*., 2000) proposed that although the ability of toludine blue to stain dysplastic tissues is believed to rely solely on quantitative differences in the amounts of DNA and RNA, it is feasible that some inherent qualitative defect may be responsible.[6]

Mashberg (1980) reported 9.2%, Onofre *et al* (2001) reported 2%, (Epstein *et al*., 2003) gave 64%, and (Zhang *et al*., 2005) reported 26% and (Siddiqui *et al*., 2006) reported 13% of false positive. False-positive associated with retention of dye in inflammatory and traumatic lesions have been extensively documented Mashberg (1981) (Niebel *et al*., 1964). (Silverman *et al*., 1984). False positive are also attributed to the experience of the clinician, and have been found to be lower for rinse technique than application technique Mashberg (1983). Mashberg (1980) suggested a 10-14 day waiting period to allow inflammatory lesions to resolve thus decreasing the false positives by 8.5%. (Siddiqui *et al*., 2006) exhibited complete resolution of false positive (13%) on second evaluation. However, such a procedure, which must be included in the protocol, is seldom practiced, and account for unwanted biopsies.[1] Also the study is subjective to interexaminer evaluation.

Allen (1998) argued against the massive commercial interest behind the use of Toludine blue testing. (Ephros and Mashberg 1999) recommended the use of Toludine blue as a diagnostic adjunct, but strongly opposed the use for mass screening programs or detection of "precancerous" lesions. Further (Kerawal *et al*., 2000) suggested the use of Toludine blue as an adjunct in identifying invasive tumour at mucosal resection margins alone, as it appears to be of no benefit in delineating carcinoma-in-situ or severe dysplasia. Unfortunately such recommendations have been neglected and Toludine blue is still being used unsatisfactorily. The authors agree with Allen (1998) that the high degree of false negative results obtained with dysplastic mucosal lesions may seriously mislead clinicians and affected patients, and there is no substitute for a thorough clinical and histopathologic examination.[4,5]

Toludine blue staining should be routinely used as a method to assist in the choice of biopsy site and in the follow-up of premalignant lesions. Further studies with more number of cases are suggested to establish these stains as sound diagnostic adjuncts.[7]

Early detection and timely intervention is the essence of any cancer treatment protocol. Supravital staining with 1% Toludine blue is useful in the early detection of malignancies. Toludine blue can be used as a supplement to clinical and histological examination so as determine the biopsy site inclinically diagnosed potentially malignant and malignant lesions. It assists in selecting the best site for biopsy. It is very useful in the developing countries like India because of the cost effectiveness and easy technique. The test is sensitive, simple, noninvasive and highly cost effective procedure.

References

1. Juhi Upadhyay, Nirmala N Rao, Ram B Upadhyay*, Pankaj Agarwal1 ; Reliability of Toluidine Blue Vital Staining in Detection of Potentially Malignant Oral Lesions - Time to Reconsider ;*Asian Pacific J Cancer Prev*, 12, 1757-1760
2. Kamarthi Nagaraju, Shiva Prasad, L Ashok;Diagnostic efficiency of toluidine blue with Lugol's iodine in oral premalignant and malignant lesions ; *Indian J Dent Res* 2010;21:218-23

3. Rosenberg D, Cretin S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. Oral Surg Oral Med Oral Pathol 1989;67:621-7.
4. 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;85:444-6.
5. Kurita H, Kurashina K. Vital staining with iodine solution in delineating the border of oral dysplastic lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;81:275-80.
6. Epstein JB, Scully C, Spinelli J. Toluidine Blue and Lugol′s Iodine application in the assessment of Oral Malignant disease and lesions at risk of malignancy. *J Oral Pathol Med* 1992;21:160-3.
7. Mahesh Chandra Hegde, Panduranga M. Kamath, Suja Shreedharan, Naveen Kumar Dannana, Ravikumar M. Raju ; SUPRAVITAL STAINING: IT'S ROLE IN DETECTING EARLY MALIGNANCIES; *Indian Journal of Otolaryngology and Head and Neck Surgery* Vol. 58, No. 1, January-March 2006.