

RESEARCH COMMUNICATION

Reliability of Toluidine Blue Vital Staining in Detection of Potentially Malignant Oral Lesions - Time to Reconsider

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Abstract

Being simple and inexpensive toluidine blue has been in use for more than two decades for the detection of potentially malignant oral lesions (PMOL's) and malignant lesions. Although there has been consensus that staining often assists in the identification of these lesions, results have been diverse. In most studies false negative were not recorded as biopsies of lesions that did not retain toluidine blue were not performed. Thus the present study attempted to evaluate the efficacy of toluidine blue vital dye for detection of PMOL's. The study included 47 biopsies (TBP:35 and TBN:12), of which 23 cases were confirmed as dysplastic (TBP=17 and TBN=6), 7 as hyperkeratosis (TBP=4 and TBN=3), 8 as epithelial hyperplasia (TBP=6 and TBN=3) and 5 as other benign lesions (TBP=4 and TBN=1). The validity test revealed a sensitivity of 73.9% and specificity of 30%. The positive predictive value was 54.8% and negative predictive value of 50%. The study intends to highlight the false negative result (26.1%) which was mainly attributed to mild dysplasia and the false positive (32.6%) which included hyperkeratosis, hyperplasia, lichen planus and traumatic ulcer. The study concludes that toluidine blue staining should not blindly direct the clinician's opinion, and strongly discourages the use of toluidine blue as a screening test and the results should be interpreted with caution.

Keywords: PMOL's, - toluidine blue vital staining - reliability

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Introduction

Oral cancer is usually 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. Toluidine blue, discovered during 1960s, is a basic metachromatic dye of thiazine group that shows affinity for the perinuclear cristernae of DNA and RNA (Herlin et al., 1983). (Allen et al., 1949) introduced the therapeutic use of toluidine chloride as an i/v anti-heparin agent. In 1960 Sherwin suggested to (Strong et al., 1968) the use of toluidine 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% toluidine chloride stain in delineation of neoplastic epithelium of the cervix. Its use in-vivo is based on the fact that dysplastic and anaplastic cells contain quantitatively 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).

However, the mechanism by which the dye differentially stains malignant and dysplastic tissues remains unclear. (Epstein et al., 2003) showed that use of toluidine blue is more sensitive than clinical examination alone, and compared to iodine staining (sensitivity of 73%), toluidine blue (sensitivity 91.2%) has yielded better results. For PMOL's the sensitivity is about 72-100% and the specificity being 45-93% (Missmann et al., 2006). Recent reports have concluded toluidine blue retention in high risk PMOL's (Zhang et al., 2005) and high-risk molecular clones, even in lesions with minimal or no dysplasia (Guo et al., 2001). However, the detection of low-grade oral dysplasia has been less consistent (Onofre et al 2001). Further there has not been any agreement on the intensity of the stain uptake, though (Gandolfo et al., 2006) suggested dark royal blue staining to be the true

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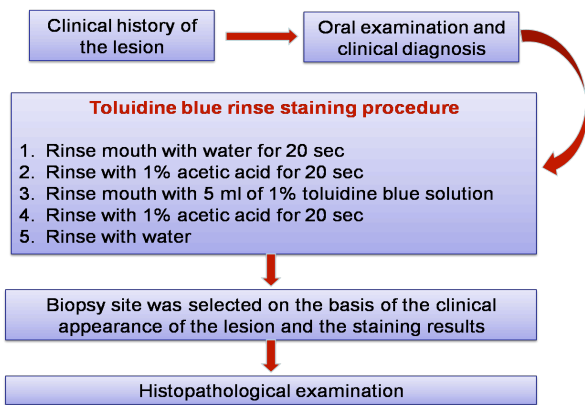


Figure 1. The Toluidine Blue Rinse Protocol followed in the Study

positive outcome of test with different histological pattern of uptake. In spite of claims from several authors for use of toluidine blue vital dye for the detection of PMOL's needs serious re-considerations. The present study was conducted to evaluate the efficacy of toluidine blue vital dye as a diagnostic adjunct and further discuss the judgment to continue its use.

Materials and Methods

The study included 47 patients visiting the Dental clinics of Manipal College of Dental Sciences, Manipal, under routine OPD, of which 21 % (n=10) were females and 79 % (n=37) males. The age of the subjects ranged from 31-75 years, with a mean age of 53.83 years. Clinically a provisional diagnosis of homogeneous Leukoplakia, speckled Leukoplakia, Erythroplakia & Erosive lichen planus (Based on the criteria proposed by WHO 2005) was established after a detailed history of the lesion and through oral examination. The subjects were then subjected to toluidine blue rinse procedure (Figure 1).

A suitable incisional/punch biopsy was obtained on the basis of site retaining the stain (Toluidine blue positive lesions, TBP). With regard to interpretation of staining, as suggested by Mashberg (15, 16) doubtful light blue stain was considered as positive until biopsy proves the contrary. Biopsy was also obtained from those lesions which did not retain any stain but were clinically suggestive of a PMOL's (Toluidine blue negative lesions, TBN). All the specimens were subjected to histopathologic examination and reviewed by two oral pathologists

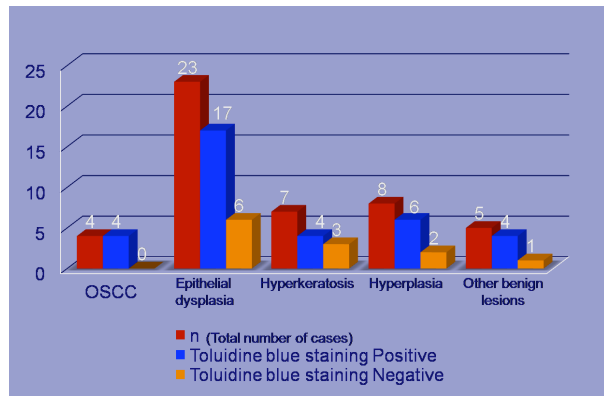


Figure 2. Comparison of Clinico-histopathologic Results and Toluidine Blue Staining

blinded to toluidine blue staining results.

The tissue specimens were histologically categorized as (a) Benign: hyperkeratosis, hyperplasia, & other non-malignant lesions, (b) Dysplasia: mild, moderate & severe dysplasia; and finally (c) Oral squamous cell carcinoma (OSCC) .

Comparison was subsequently made between the histopathologic patterns and the presence or the absence of staining. Diagnostic validity test, i.e. sensitivity, specificity, and the positive and negative predictive results were performed according to the method proposed by (Rosenberg and Cretin 1989), and false negative and false positive were determined

Results

Differences in test characteristic between clinical examination, toluidine blue application and histopathologic interpretation were evident. Of 47 biopsies (TBP=35; TBN=12) obtained, 4 TBP cases were histopathologically diagnosed as OSCC and were excluded from the study. 23 cases were confirmed as dysplastic (TBP=17;TBN=6), 7 as hyperkeratosis(TBP=4;TBN=3), 8 as epithelial hyperplasia(TBP=6;TBN=3) and 5 as other benign lesions(TBP=4;TBN=1) (Figure 2).

Toluidine blue staining gave a sensitivity of 73.9% and specificity of 30%. The positive predictive value was 54.83and negative predictive value of 50%. The present study intends to highlight the false negative (26.08%) which was mainly attributed to mild dysplasia and the false positive (32.6%) which included hyperkeratosis, hyperplasia, lichen planus and traumatic ulcer (Table 1).

Table 1. Discrepancies between Clinical and Histopathologic Diagnosis and Correlation with Results of Toluidine Blue Staining

HISTOPATHOLOGIC DIAGNOSIS	CLINICAL DIAGNOSIS						Total (%)	TOLUIDINE BLUE	
	Homogeneous*	Verrucous*	Speckled*	Erythroplakia	ELP	NHU		Positive	Negative
OSCC	-	3	-	-	1	-	4 (8.51)	4	-
Dysplasia	16	1	3	2	-	1	23 (49.9)	17	6
Hyperkeratosis	4	-	1	1	1	-	7 (14.9)	4	3
Hyperplasia	6	-	-	1	1	-	8 (17.0)	6	2
Other benign lesion	3	-	-	1	-	1	5 (10.6)	4	1
Total	29	4	4	5	3	2	n=47	35	12

Sensitivity (Dysplasia) 73.9; + ve PV 54.8; Specificity 30; - ve PV 50; false negative 26.1%; false positive 32.6%

*leukoplakia; ELP, erosive lichen planus; NHU, non-healing ulcer PV, predictive value

Discussion

Literature holds sufficient reports regarding the sensitivity and specificity of toluidine blue for detection of PMOLs, however it seldom highlights the false negative and false positive results which are equally significant. It is this false negative and false positive which the general practitioners are blinded to and have encouraged indiscriminate use of toluidine blue staining as the most popular screening/detection method.

The present study reports a false negative result of 26.08% in accordance with (Warnakulasurya & Johnson 1996) (26%). 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%. In most of the studies including the present study, the false negative was mainly attributed by low grade dysplasia. 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). Toluidine 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 toluidine application in the detection of dysplastic lesions (Epstein et al., 1997). (Kerawala et al., 2000) proposed that although the ability of toluidine 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.

Further the present study observed a false positive of 32.55% which was mainly attributed to epithelial hyperplasia and hyperkeratotic lesions. 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) (Nebel 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.

Since the dye is known to react with ribonucleic acid, Wysocki (1999) also described a possible mutagenic effect of toluidine blue, especially when vitally stained cells are exposed to high energy irradiation, e.g. light.

Allen (1998) further argued against the massive commercial interest behind the use of toluidine blue testing. (Ephros and Mashberg 1999) recommended the use of toluidine 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 toluidine 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 toluidine blue is still being used unsatisfactorily. Moreover, most of the studies claiming reliability of toluidine blue stains have been conducted in specialized cancer centres, thus it is suggested to expand the scenario for general dental practice beyond experienced and trained clinicians. 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.

In conclusion, toluidine blue application is not a valuable adjunct and its use needs serious reconsideration. And supporting the views of (Epstein et al., 1997) the present study strongly discourages the use of toluidine blue as a screening test; furthermore the results should be interpreted with caution.

Acknowledgements

The authors declare that there is no conflict of interest with this work.

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