Original Research Paper

Volume-8 | Issue-7 | July-2018 | PRINT ISSN No 2249-555X

Pathology

THE UTILITY OF TOLUIDINE BLUE AS A DIAGNOSTIC ADJUNCT IN DYSPLASTIC ORAL LICHEN PLANUS

S K Padmakumar — Associate Professor & MDS in the Department Of Oral Pathology and Microbiology, Government Dental College, Thiruvananthapuram.

Sivakumar R — Assistant Professor in the Department Of Oral Pathology and Microbiology, Government Dental College, Thiruvananthapuram.

Renu Chandran M* — Senior Resident & MDS in the Department Of Oral Pathology and Microbiology, Government Dental College, Thiruvananthapuram.*Corresponding Author

Alex K Varghese — Post Graduate student in the Department Of Oral Pathology and Microbiology, Government Dental College, Thiruvananthapuram.

ABSTRACT Although oral lichen planus is considered as a potentially malignant disorder, still there are controversies regarding its malignant transformation. The actual overall frequency of malignant transformation is low, varying between 0.3 and 3%. Histopathology is considered as the gold standard for the diagnosis of dysplastic OLP. Toluidine blue is a basic metachromatic stain which shows affinity for DNA and RNA. 30 patients were selected who were clinically suspicious of dysplastic OLP. Biopsy was taken to correlate the clinical and histological features. The association of biopsy findings and percentage of detection was tested statistically and was found highly significant ($p > .001$). Sensitivity and specificity were estimated as 90% and 100% respectively. Accuracy was found to be 92.5%.

KEYWORDS : Dysplastic Oral Lichen Planus, Toluidine Blue, Vital Staining

INTRODUCTION

Oral lichen planus (OLP) is a common muco cutaneous disease, first described by Wilson in 1869. Lichens are primitive plant that consists of symbiotic algae and fungi and the word '*planus*' in Latin means flat [(1)]. The prevalence of the disease ranges between 0.5% and 2.6% and the range of malignant transformation varies between 0% and 10% [(2).] The etiology of OLP is proposed to be immune mediated. Various types of OLP showed a varied rate of malignant transformation [(3)]. The highest rate of malignant transformation has been noted in erythematous and erosive type.[(4)] The oral lichenoid reaction is considered a variant of the disease that needs to be clearly diagnosed as a separate entity from OLP and treated [(5)].

Histopathology is considered as the gold standard for the diagnosis of dysplastic OLP. Vital staining for detection of dysplasia has been applied in oral setting for over 50 years by means of the dye Toluidine blue (TB)[(6)].

Toluidine blue is a basic metachromatic stain which shows affinity for DNA and RNA. By virtue of the fact that cancer cell contain quantitatively more DNA and RNA than normal epithelial cell, toluidine blue delineates the area of malignancy. Vital staining using 1% TB to assess its utility as a diagnostic aid in dysplastic lesion is done in the present study.

Aims and objectives

To evaluate the utility of 1% TB dye application as an aid to the recognition and diagnosis of dysplastic lesions in clinically evident cases of OLP.

Materials and methods

The sample was obtained from Government Dental College Thiruvananthapuram. 30 patients with oral lesions clinically suggestive of OLP was selected. Control group consist of 10 persons with no clinical signs of oral mucosal lesions.

Rinse mouth with water for 20 seconds to remove debris,followed by rinse with 1% acetic acid for 20 seconds to remove saliva. Apply 1% TB solution to lesion .Wait for 2 minutes and rinse with 1% acetic acid for 1 minute to clear excess stain. A stain is considered positive for malignancy and dysplastic lesions if the lesion stain dark blue. Occasional equivocal stains are considered positive. A negative stain generally implies no absorption of the stain .

Biopsy of stained area was performed after obtaining consent. Then the specimens were placed immediately in 1% neutral formalin Tissue sectioned and stained with hematoxylin and eosin.

The findings of the histologic examination were broadly divided into various histological criteria delineated by Hedburg N and Hunter N [(7)]. The histological parameters were graded from – to +++.

Results

Biopsy was taken to correlate the clinical and histological features. Reticular type of lesions is the largest with 30% among all the lesions followed by erosive type of lesions. Majority of stain reaction was equivocal(Table 1)

Table 1: Distribution according to staining reaction

Stain reaction	Number	Percentage
Positive	11	27.5
Equivocal	16	40
Negative	13	32.5

The association of biopsy findings and percentage of detection was tested statistically and was found highly significant. Thus it was inferred that the detection of dysplasia was quite probable in maximum number of cases (75%) followed by keratosis (52.5%) (Table 2).

Table 2: Distribution according to biopsy findings

Biopsy findings	Detected	Not detected	Total 40
Keratosis	52.5%	47.5%	40
Acanthosis	40%	60%	40
Dysplasia	75%	25%	40

Out of the 11 positive cases, 9% were severe and the remaining 91% happened to be of moderate dysplasia. The maximum dysplasia cases were detected among positive stain reaction followed by equivocal. At the same time 67% dysplasia cases were mild amongthose who have negative stain reaction. Statistical test revealed no association, probably due to small sample size (Table 3).

Table 3: association between stain reaction and severity of dysplasia detected cases

Stain reaction	Severity of dysplasia detected cases						Total
	Mild(+)		Moderate(++)		Severe +++		
	No.	%	No.	%	No.	%	
Positive	0	-	9	90	1	10	10
Equivocal	6	35	10	59	1	6	17
Negative	2	67	1	33	0	-	13

Sensitivity and specificity were estimated as 90% and 100% respectively. Accuracy was found to be 92.5%.

Discussion

The current Working Group of WHO (2005) did not favour

subdividing pre cancer to lesions and conditions and the consensus view was to refer to all clinical presentations that carry a risk of cancer under the term 'potentially malignant disorders' to reflect their widespread anatomical distribution [8]. Prevalence of OLP varies from 0.5% to 3% [9]. Malignant transformation of OLP is still a controversy [10-14]. In a 7 year follow-up study the annual malignant transformation rate amounted less than 0.5% [16]

TB is an acidophilic dye of thiazine group which selectively stains acidic tissue components. Since 1960s it has been used as vital staining in vivo based on the fact that dysplastic and anaplastic cells contain quantitatively more nucleic acid than normal. Also the intercellular canals in the malignant epithelial cells are wider than normal causing more penetration of the dye [17]. But still there is some controversies regarding the efficacy of toluidine blue vital staining as an indicator of premalignant and malignant lesions of oral cavity.

Early studies have shown that carcinoma and carcinoma in situ retain a dark blue stain following TB rinse [18] which was confirmed by the present study. The clinical impression gained was that a dark blue colouration of mucosa which is resistant to decolourisation with 1% acetic acid post rinse is strongly indicative of a severe dysplasia. Unlike malignancies, precancerous lesions yield a variety of colour reactions. In a study conducted by Warnakulasuriya et al, 74% of dysplasia cases had stained positive [19]. Mashberg, following extensive studies claims that the lesion with limited dysplasia do not stain with TB consistently [20].

Some studies have shown that sensitivity of TB test in the detection of malignant change ranges from 86% - 100% and specificity ranged from 63-94 % [19]. In this study of dysplastic OLP, sensitivity is found to be 90% and specificity was 100% with an accuracy of 92.5%. The lowest specificity recorded was 50 [21]. Test accuracy measures the agreement between the test and the gold standard. In the study done by Epstein J B et al, the test accuracy was 67% which was less as compared to the present study.

False positive retention of dye in non-dysplastic conditions has been reported with varying frequency. In a study done by Mashberg et al, recorded a false positive rate of 8.5% [22] . In the present study it was 0. It may be due to the fact that the dye was applied on patients after 2-3 weeks, while giving instructions to discontinue the habit so that false positivity due to ulceration and inflammation can be minimised.

In the present study false negativity was found to be 7.5%. A study done by Mashberg et al, showed a false negativity of 4.8% which was found to be less than the value obtained in the present study. This variability may be due to the fact that dysplastic epithelium including mild, moderate and severe cases were considered in the present study. The great value of toluidine blue staining lies in its control over false negative clinical findings that is it detects the lesions too subtle to be clinically appreciated.

Conclusion

TB application is an immediate, easy and valuable adjunct in the clinical examination of dysplastic oral lesions. In this present study, the accuracy and sensitivity of TB in detecting OLP was found to be good. The feasibility of the techniques as a screening tool still needs further evaluation. It should be stressed that although a lesion stains with TB or meets the visual criteria for dysplastic lesion, biopsy is mandatory.

References

1. Neville BW, Damm DD, Allen CM, Bouquet JE. 2nd ed. Philadelphia: W. B Saunders; 2002. Oral and Maxillofacial Pathology; pp. 680–7.
2. Irani, Soussan, Alireza Monsef Esfahani, and Anahita Ghorbani. "Dysplastic change rate in cases of oral lichen planus: A retrospective study of 112 cases in an Iranian population." Journal of oral and maxillofacial pathology: JOMFP 20.3 (2016): 395.
3. Krupaa, R. Jayasri, et al. "Oral lichen planus: An overview." Journal of pharmacy & bioallied sciences 7.Suppl 1 (2015): S158.
4. Ismail, Sumairi B., Satish KS Kumar, and Rosnah B. Zain. "Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation." Journal of oral science 49.2 (2007): 89-106.
5. Irani, Soussan, et al. "Detection of Helicobacter pylori in oral lichen planus and oral lichenoid reaction." Avicenna Journal of Dental Research 6.2 (2014).
6. Niebel, H. H., and B. Chomet. "In Vivo Staining Test for Delineation of Oral Intraepithelial Neoplastic Change: Preliminary Report." Journal of the American Dental Association (1939) 68 (1964): 801.
7. Hedberg, N., A. Ng, and N. Hunter. "A semi-quantitative assessment of the histopathology of oral lichen planus." Journal of Oral Pathology & Medicine 15.5 (1986): 268-272.
8. Warnakulasuriya, S., Newell Johnson, and I. Van der Waal. "Nomenclature and classification of potentially malignant disorders of the oral mucosa." Journal of oral pathology & medicine 36.10 (2007): 575-580.
9. Farhi, David, and Nicolas Dupin. "Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: facts and controversies." Clinics in dermatology 28.1 (2010): 100-108.
10. Krutchkoff, David J., Leslie Cutler, and Stella Laskowski. "Oral lichen planus." Journal of Oral Pathology & Medicine 7.1 (1978): 1-7.
11. Voûte, A. B. E., et al. "Possible premalignant character of oral lichen planus the Amsterdam experience." Journal of oral pathology & medicine 21.7 (1992): 326-329.
12. Eisenberg, Ellen. "Oral lichen planus: a benign lesion." Journal of oral and maxillofacial surgery 58.11 (2000): 1278-1285.
13. Van der Meij, E. H., H. Mast, and Isaäc van der Waal. "The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective five-year follow-up study of 192 patients." Oral Oncology 43.8 (2007): 742-748.
14. Accurso, Brent T., et al. "Allelic imbalance in oral lichen planus and assessment of its classification as a premalignant condition." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 112.3 (2011): 359-366.
15. Poomsawat, Sopee, et al. "Overexpression of cdk4 and p16 in oral lichen planus supports the concept of premalignancy." Journal of Oral Pathology & Medicine 40.4 (2011): 294-299.
16. Bombeccari, Gian Paolo, et al. "Oral lichen planus and malignant transformation: a longitudinal cohort study." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 112.3 (2011): 328-334.
17. Richart, Ralph M. "A clinical staining test for the in vivo delineation of dysplasia and carcinoma in situ." American Journal of Obstetrics & Gynecology 86.6 (1963): 709-712.
18. Niebel, Harold H., and Bernhard Chomet. "Staining test for delineation of oral intraepithelial neoplasms: In Vivo Staining Test for Delineation of Oral Intraepithelial Neoplastic Change: Preliminary Report." The Journal of the American Dental Association 68.6 (1964): 801-806.
19. Warnakulasuriya, K. A. A. S., and Newell W. Johnson. "Sensitivity and specificity of OraScan® toluidine blue mouthrinse in the detection of oral cancer and precancer." Journal of oral pathology & medicine 25.3 (1996): 97-103.
20. Mashberg, Arthur. "Tolonium (Toluidine Blue) Rinse\p= m-\A Screening." Jama 245 (1981): 2408-2410.
21. Rosen, I. B., M. Cornish, and J. Edelson. "Detection of early oral cancer by toluidine blue." Journal of the Canadian Dental Association 37.9 (1971): 347-349.
22. Mashberg, Arthur. "Reevaluation of toluidine blue application as a diagnostic adjunct in the detection of asymptomatic oral squamous carcinoma: a continuing prospective study of oral cancer III." Cancer 46.4 (1980): 758-76