Heal Talk

Demonstration of Toluidine Blue in Oral Mucosal Lesions

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Abstract

Early detection of cancer is of prime Importance. It helps us to reduce morbidity and mortality. 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[1,2]. Despite the easy accessibility of the oral cavity during physical examination, many malignancies are not diagnosed until late stages of disease. Clinical diagnosis of oral squamous cell carcinomas (OSCC) is not difficult when the lesion is obviously invasive or functional limitation is present[3]. Supravital stain Toluidine Blue (TB) has been used to mark the area for biopsy and to mark the full extent of premalignant lesion. It has been reported that toluidine blue stains premalignant and malignant lesions, but, not the benign lesions and normal mucosa[4].Toludine blue,discovered during 1960s, is a basic metachromatic dye of thiazine group that shows affinity for the perinuclear cristernae of DNA and RNA introduced the therapeutic use of tolonium chloride as an i/v anti-heparin agent[5].

Aims And Objective: To assess the reliability of toluidine blue as clinical screening test, for detection of presence of epithelial dysplasia in oral mucosal lesions.

Study Design: Study Group consisted of 56 patients with lesions on the oral buccal mucosa. After rinsing with distilled water and the lesion was swabbed with 1% acetic acid for 30 seconds than with toluidine blue for 20sec, followed by rinsing with distilled water and application of 1% acetic acid for 30sec, again rinsed with distilled water. Biopsy was taken from toludine blue positive lesions and graded for epithelial dysplasia to analyze the role of toludine blue staining in selecting lesions requiring biopsy.

Conclusion: Staining with toluidine blue was demonstrated to be highly reliable in the detection of dysplasia because false-negative results for the lesions did not occur. Toluidine blue staining is an adjunct to clinical judgment and not a substitute for either judgment or biopsy.

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Introduction

It is of great importance and neccesity for the development of visual aids that will facilitate the detection of oral premalignant lesions (OPLs) with a high-risk of progression. Early detection of OPLs is central to the improvement of this prognosis. Even when OPLs are identified, our ability to predict outcome is a challenge because the majority of OPLs will not progress.[6] The location and appearance of oral lesions does not always facilitate early detection. However appearance shows that most lesions are diagnosed only after becoming symptomatic.Clinicians may fail to recognize patients at high risk of developing oral cancer and the appearance of early sequences carcinoma in the oral cavity can easily be overlooked.[6] Despite good prospects of early diagnosis small developing lesions often go unrecognized by clinicians. 60% of lesions are well advanced by the time of discovery[7].Vital tissue staining has been identified as an adjunct to the early recognition of malignant lesions. Toluidine blue {TB} (tolonium chloride) is a member of thiazine group of metachromatic dyes. TB staining may appear as a Dark Royal Blue or a Pale Royal Blue color.[8]

In this study, biopsy was performed on the 56 patients with primary OPLs to relate their toluidine blue status to outcome as well as to conventional histopathologic features.

Material & Methods

The study group consisted of 56 patients having oral lesions suspicious of premalignancy. These included patients with Homogenous Leukoplakia (23), Nonhomogeneous Leukoplakia (18), Oral Submucous Fibrosis (4), Erythroplakia (6), and Tobacco pouch keratosis (5). Only those patients who consented for incisional biopsy were included in the study. The exclusion criteria includes, patients with lesions at sites where application of toloudine blue or biopsy was not possible, patients with systemic disorders, oral submucous fibrosis patients with severe fibrosis or trismus, patients not willing for biopsy. Traumatic lesions and oral inflammatory changes were excluded from the study to avoid false positive results. Subjects were selected from both sexes and all age groups. Informed consent was taken from patients before application of the vital stain and biopsy.

Composition of Toluidine Blue stain 100 c.c of the 1% TB solution: consists of 1 gm of TB powder, 10 ml of 1% acetic acid, 4ml of absolute alcohol and 86ml of distilled water to make up 100 ml of a 1% solution of TB. The ph of the solution is adjusted to 4.5 [9].

Technique of Staining:

Mucosal surfaces were stained preoperatively. Initially the patient is asked to rinse his mouth twice with water for 20 seconds to remove the debris. Next 1% acetic acid was applied for 20 seconds to remove any ropey saliva and remaining organic debris using cotton swab. 1% TB solution is then applied for 20 seconds with cotton Swab. Followed by application of 1% acetic acid again on mucosa attained by toluidine blue by cotton swab to remove nonspecific mechanically retained stain from the lesion. Finally patient was asked to rinse mouth with water and then toluidine blue staining was assessed(Fig 1&2).Biopsies were performed from both toluidine blue positive as well as toluidine blue negative lesions with patients consent. All specimens were subjected to formalin fixation & hemotoxylin & eosin processing.(Fig 3&4)

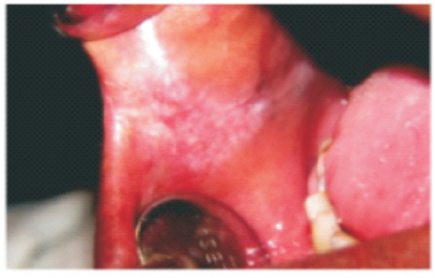

Fig 1: lesion over buccal mucosa

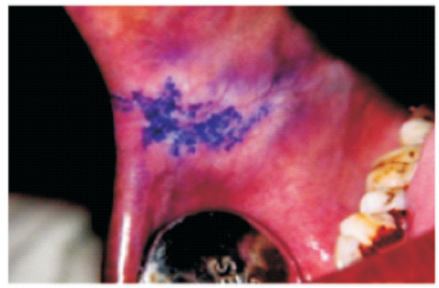

Fig 2: lesion after staining with TB

Results

Most of the patients were in the age group of 35 - 65 years with a mean age of 47 years. In our study a male preponderance was seen and majority of our patients had more than one risk factor predisposing for oral cancer, that included smoking, alcohol, quid/ tobacco chewing, and spicy food. Buccal mucosa was the most common site of lesion with 45 cases (80.3%) followed by labial vestibule in 8 cases (14.2%) and commisure of lip 3 cases (5.3%).

Overall 51 cases (91.07%) stained positive with toluidine blue vital stain. These cases when subjected to biopsy, 46 (90.1%) were positive for dysplasia and 5 (9.8%) were nondysplastic histologically. 5 cases which stained positive but were negative for dysplasia on biopsy, constituting false positives, included three cases of tobacco pouch keratosis, and one each of homogeneous leukoplakia and OSMF . 5 cases (8.9%) which did not take up the stain clinically included two cases of OSMF and one each of homogeneous leukoplakia, nonhomogeneous leukoplakia, and erythroplakia. Out of these five negative cases only the two OSMF cases were found negative for dysplasia on histopathological examination (true negative,) while remaining showed epithelial dysplasia (false negative).

For Non homogenous leukoplakia our study group consisted 18 cases. Out of which 16 were stained toluidine blue positive. All these 16 cases were found dysplastic on

histopathological examination while two case which did not take up stain clinically was also found to be histologically dysplastic. Since there were no true negative cases

Another group constitutes 6 cases of erythroplakia. Out of it 5 cases took up toluidine blue stain clinically and were dysplastic when examined histopathologically. One case neither took stain and nor was dysplastic when examined histologically.

Out of 23 cases of homogeneous leukoplakia 22 stained positive with toluidine blue out of which 20 cases were found to be dysplastic on histopathological examination and 2 case were nondysplastic . One case which did not clinically take up the stain was found to be histologically dysplastic while there was no true negative case in this group.

Next group consisted of 5 cases of tobacco pouch keratosis. All the cases were positively stained with toluidine blue clinically. On histopathological examination it was found that only 2 out 5 cases were dysplastic and 3 cases were non dysplastic.

There were 4 cases of OSMF and out of which 2 cases took up toluidine blue stain and 2 cases did not take stain clinically. When analyzed histopathologically it was found that only 1 case was dysplastic and rest 3 cases were nondysplastic.(Table 1)

Lesions	N	True Positive	True Negative	False Positive	False Negative
Homogenous Leukoplakia	23	21	-	1	1
Non Homogenous Leukoplakia	18	16	-	-	2
Erythro-plakia	6	5	-	-	1
Oral sub mucous fibrosis	4	1	2	1	-
Tobacco pouch keratosis	5	2	-	3	-
Total	56	45	2	5	4

Table 1 The results of individual lesions are as follow

Histolological Impression:

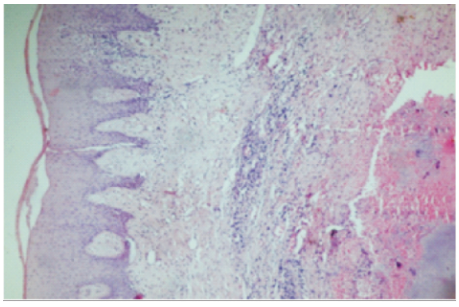

Fig 3: Non Dysplastic Epithelium

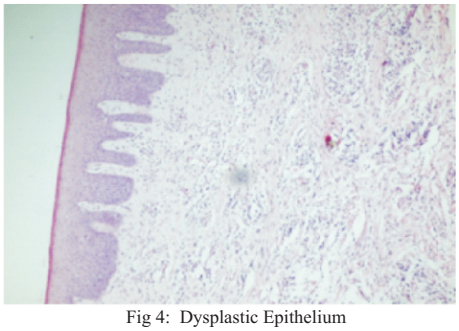

Fig 4: Dysplastic Epithelium

Discussion

Oral cancers constitute 40% of all cancers and rank as the most common cancer in men and third most common cancer in women in India.[10] Early detection of oral cavity carcinoma is, however, far from straightforward. Presence of precancerous lesions is not easy to detect due to a high likelihood of false-positivity. Histopathology continues to be used as the reference standard test.[11] Toluidine blue staining is considered to be a sensitive adjunct tool for identifying early oral SCC and high-grade dysplasias. Toluidine blue was first used by Riechart in 1963 to stain uterine cervical carcinoma in Situ. Its use in vivo is based on the fact that dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissues. In addition, malignant epithelium may contain intracellular canals that are wider than normal epithelium; this is a factor that would enhance penetration of the dye.[12] The results of our study shows, toluidine blue when used as a vital stain is highly sensitive in identifying oral lesions with dysplastic epithelium. Out of total of 49 lesions which were diagnosed positive for presence of dysplasia by histological examination 45 stained positively with toluidine blue. These findings are in concordance with previous studies by Warnaku lasuriya, Arthur Mashberg and Epstein J B.[9,13,14]

5 lesions which were positive on staining were found to be histologically negative. Out of these 3 were tobacco pouch keratosis, and 1 case of homogenous leukoplakia and one cases of OSMF. It has been proposed that in certain lesions the premalignat changes might have started at molecular level but may not have been expressed at light microscopic levels yet. In fact Guo et al have shown that a group of lesions showing false positivity with toluidine blue have shown allelic losses and loss of heterozygosity.[15]

Hence it may be assumed that some of the false positive lesions may represent lesions which are actually positive at molecular level. Hence molecular study of these lesions may help us in resolving the nature of these false positive lesions. Another suggestion for reducing the false positive cases have been by M. Eslami et al who stated that false positive lesions should be reviewed after 7 to 14 days by second staining, thus allowing time for healing of traumatic or inflammatory as these lesions have shown tendency to take up the stain.[16]

The reasons for false negative staining are not very well understood. In cases of leukoplakia and other lesions with hyperkeratotic epithelium,[16,17] it could be possible that thick, impermeable layer of keratin may prevent penetration of the dye to deeper layers which usually show nucleic acid changes hence causing false negative staining. Another reason which has been suggested is that there may be variability in assessing the staining by different observers[18], leading to different definition of false negative staining. Eventhough this might be a limitation, in our study we found that the rate of false negative was quite low hence may not be a limitation in the utility of the stain.

Conclusion

Early detection and timely intervention is the essence of any cancer treatment protocol. Supravital staining with 1% toluidine blue is useful in the early detection of malignancies.[19] We felt that it is an appropriate adjunct to visual examination, especially for patients with suspicious lesions. So in such cases referral to a centre or individual with extensive experience in head and neck cancer is recommended.

References

1. Silverman S Jr, Bhargava K, Smith LW, et al . Malignant transformation and natural history of oral leukoplakia in 57,518 industrial workers of Gujarat, India. Cancer, 197638,1790-5.
2. Pindborg JJ, Daptary DL, Mehta FS . A follow-up study of 61 oral dysplastic precancerous lesions in Indian villages.Oral Surg Oral Med Oral Pathol 1977;43:383-90.
3. Siddiqui I, Farooq U, Rafi T. Role of Toluidine Blue in early detection of oral cancer. J Med Sci 2006;22:184-7.
4. Gupta A, Singh M, Ibrahim R, Mehrota R. Utility of toluidine blue staining and brush biopsy in precancerous and cancerous oral lesions. Acta Cytol 2007;51:788-94.
5. Herlin R Marnay J, Jacob JH, Ollivier JM, et al.. A study of the mechanism of staining of the toluidine blue dye test.1983 Endoscopy; 15: 4-7.
6. Zhang L,Williams M. Toluidine Blue Staining Identifies High-Risk Primary Oral premaignant lesions with poor outcome.Epidemiology and prevention,2005.American Association for cancer research.
7. Mashberg A, Samit. Early Detection, Diagnosis and Management or oral and oropoharyngeal cancer. A Cancer Journal for Clinicals 1989; 39: 67.
8. Sergio Gandolfo, Toluidine blue uptake in potentially malignant oral lesions in vivo: clinical & histological assessment. Oral Oncology 2006: 42, 89-95
9. Mashberg A. 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 1980;46:758-63.
10. Mehrotra R, Singh M, Thomas S, Nair P, Pandya S, Nigam NS, Shukla P. 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(2):151–156.
11. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. J Am Dent Assoc.1999;130(10):1445–1457.
12. Richart RM: A clinical staining test for the in vivo delineation of dysplasia and carcinoma in situ. Am J Obstet Gynecol. 1963, 86: 703-712.
13. Warnakulsuriya, Johnson NW. Sensitivity and specificity of OraScan toluidine blue mouth rinse in the detection of oral cancer and precancer. J Oral Pathol Med 1996;25:97-103.
14. Epstein JB, Scully C, Spinelli JJ. 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;22:160-3.
15. Guo Z, Allelic Losses in OraTest-directed Biopsies of Patients with Prior Upper Aerodigestive Tract Malignancy. Clinical Cancer Research; Vol. 7, 1963–1968, July 2001
16. M. Eslami, Comparison and Evaluation of the Reliability of Oratest and Generic Toluidine Blue in the Detection of Oral Malignancy and Premalignancy. Journal of . Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran 2005; Vol: 2, No.3.
17. Sayed M.Tobacco-Associated Lesions of the Oral Cavity: Part I. Nonmalignant Lesions.J Can Dent Assoc 2000; 66:252-6.
18. Lauren L. Adjunctive techniques for oral cancer examination and lesion diagnosis. A systematic review of the literature. JADA, 2008; 139.
19. Joel B. Epstein, Advances in the Diagnosis of Oral Premalignant and Malignant Lesions, J Can Dent Assoc 2002; 68:617-21.