Research Article

Efficacy of Rose Bengal in comparison with Toluidine Blue in detection of potentially malignant lesions: a preliminary study

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Abstract: Introduction: Oral cancer is usually diagnosed when it becomes symptomatic and approximately two thirds of the patients present with the advanced disease, regional metastasis and consequently poor prognosis. The risk of development of carcinoma within an area of premalignant lesion is 5 times higher than in those without lesion. Malignant transformation has been reported in 43% of dysplastic leukoplakia cases. It is therefore important to identify these lesions early, for early management.

Aim: To compare the diagnostic efficiency of Rose Bengal (RB) with Toluidine blue (TB) in detecting the biopsy sites and thus establish an accurate diagnosis in detecting oral potentially malignant disorders.

Materials and Methods: In our study 27 patients with 41 lesions were included. Since one patient had not quit the habit in the two weeks following initial examination and another patient lesion disappeared in the waiting period, 2 patients (3 lesions) were not included in the study. After initial examination they were subjected to Rose Bengal and Toluidine blue stain. Positively stained lesions were subjected to biopsy and assessed for dysplasia. All the data were tabulated and statistically analysed in SPSS software version v.22.IBM, corp. Chi square test was done to compare the difference between two stains.

Results: When compared with TB staining RB showed 100% Sensitivity, 90% specificity, 96.6% positive predictive value and 100% negative predictive value.

Conclusion: Based on the results and other observations made during the study it could be suggested that RB could be used as an alternative to TB clinically. Further studies with large samples are recommended to support the evidence of this study.

Keywords :……….

Introduction

In early 1805 the concept of 'precancer' was proposed. Since then it has been well-established by researchers that a possible time lag exists between the development of earliest tissue change and its malignant transformation. This time lag gave the healthcare professional a chance to effectively intervene and prevent the process, thereby reducing cancer associated mortality and morbidity.[1] The malignant potential of these precancerous oral lesions cannot be accurately predicted solely on the basis of their clinical characteristics making histologic evaluation necessary. Histologic evaluation needs a trained health-care provider and is considered invasive, painful, expensive and time consuming. This limitation of biopsy has led to the development of other major modalities which are designed to facilitate the oral cavity examination. Recent clinical diagnostic tools for early detection of dysplasia of oral mucosa include Tolonium chloride or Toluidine blue dye, Oral CDx brush biopsy kits, salivary diagnostics and lastly optical imaging systems. [2]

Toluidine blue staining is the most common technique used for the early detection of dysplastic changes in patients with premalignant lesions. But, studies have shown as high as 30% risk of false-positive staining. [3]

Rose Bengal (RB), the 4,5,6,7-tetrachloro-2,4,5-tetraiododerivative of fluorescein, has been widely used to diagnose various ocular surface disorders including delineation of the extent of corneal and conjunctival neoplasms. [4] It has been believed to stain desquamated ocular epithelial cells, dead or degenerated cells, or wherever there is poor protection of the surface epithelium by the preocular tear film rather than lack of cell vitality. [4] These characteristic features of RB lead the researchers to apply it in potentially malignant disorders (PMDs). In none of the studies, reliability of RB stains was not compared with existing or previously practiced methods in oral PMDs. Hence the aim of this study was to compare the RB and TB stain and for early detection of dysplasia in oral PMDs.
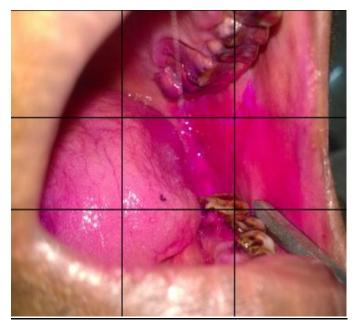
Materials and Methods:

This was a comparative study conducted for the duration of one year from mar 2015 to dec 2016. Patients who had any red&/or white lesion suspicious of PMDs and who consented to participating in the study were included. The study group consists of 25 patients 41 oral PMDs (both unilateral and bilateral) visiting the Department of Oral Medicine and Radiology of The Oxford Dental College and Hospital, Bangalore. Patients with bleeding disorders and other systemic diseases were excluded from the study.To perform the present study, ethical clearance was obtained from the Institutional Ethical Board. Study procedure was explained and informed consent was taken from the selected patients with PMDs. The patients with lesions were subjected to detailed case history, intra oral examination and photographs of the lesions were recorded. Patients with habits (chewing/smoking tobacco) were counseled to quit the habit and recalled after 2 weeks for staining. Since one patient had not quit the habit in the two weeks from the first visit, in another patient the lesion disappeared in two weeks hence, 3 lesions were not included in the study. So the total sample size was reduced 38 lesions.

Initially patients were asked to rinse their mouth with distilled water for 1 minute. 1% RB solution was applied with a cotton tip for 2 minutes. Again patients were asked to rinse their mouth for 1 minute with distilled water to remove excess RB solution and the area which had taken up the stain was photographed. Following this, patients were asked to rinse their mouth with 1% acetic acid for 1 min to remove the remaining RB stain from the lesion and were prepared for TB staining. 1% Toluidine blue was applied over the same lesion and after 30 seconds patients were made to swish with 1% acetic acid and the area stained was photographed.

These two photographs were assessed and if the stained area was similar in both the procedures single biopsy were taken. If the stained areas were different two different biopsies were taken. (Figure 1, 2, 3)

Figure 1: Lesion stained with Rose Bengal

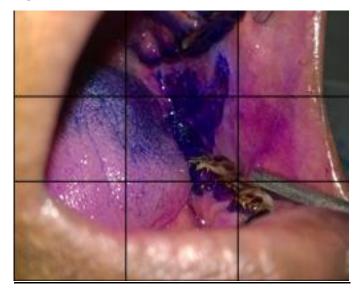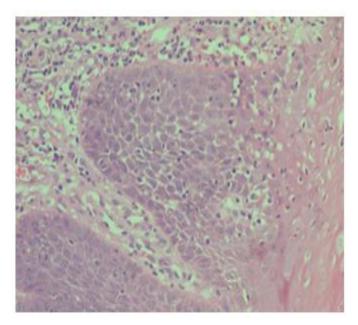**Figure 2: Lesion stained with Toluidine Blue**

Figure 3: Figure 3- Histopathologic picture showing hyperkeratosis with dysplasia (H&E staining and 20x magnification)

Statistical analysis

All the data required for this study were collected and analysed statistically to determine the significance of different parameters by using SPSS software version v.22.IBM, corp.

Comparison between TB and the RB groups were made using Chi square test and the p-value of less than 0.05 was considered statistically significant.

Results

The study group consisted of 84.2% lesions (n=32) in males (n=22 patients i.e. 88%) and 15.8% lesions (6 lesions) females (n=3 patients i.e. 12%). Out of 38 lesions diagnosed based on clinical criteria, 33 were leukoplakia, 4 lichen planus, and 1 squamous cell carcinoma (SCC). SCC was considered as negative control. The mean age of patients ranged from 38.16+/-9.6. (Table 1 and Table 2)

Table 1- Demography profile of study participants

Variables	Sub-Groups	Number	Percentage (%)
AGE (Years)	10-20	1	2.6
	21-30	6	15.8
	31-40	17	44.7
	41-50	13	34.2
	51-60	1	2.6
Mean Age	38.16 +/- 9.6		
Gender	MALE	32	84.2
	FEMALE	6	15.8

Table 2- Descriptive characteristics of study participants

Variables	Sub-Groups	Number	Percentage (%)
Habits	Present	36	94.7
	Absent	2	5.3
Location	Buccal mucosa	31	81.6
	Labial mucosa	5	13.2
	Tongue	1	2.6
	Gingiva	1	2.6
Diagnosis	Leukoplakia	33	86.8
	Lichen planus	4	10.5
	SCC	1	2.6
Rose Bengal Stain	Positive	29	76.3
	Negative	9	23.7
Toluidine Blue	Positive	28	73.7
	Negative	10	26.3
Biopsy	Done	21	55.3
	Not done	17	44.7
Histology	Dysplasia present	5	13.2
	Dysplasia absent	15	39.5
	Squamous cell carcinoma	1	2.6

In comparison with TB, RB showed 100% Sensitivity, 90% specificity, 96.6% positive predictive value negative predictive value 100% (Table 3).

Table 3- Sensitivity, specificity, positive and negative predictive value for Rose Bengal stain

Sensitivity, specificity, positive and negative predictive value for Rose Bengal stain			Toluidine blue stain		Total
			positive	negative	
Rose Bengal stain	positive	Count	28	1	29
		% within Rose Bengal stain	96.6%	3.4%	100.0%
		% within Toluidine blue stain	100.0%	10.0%	76.3%
	negative	Count	0	9	9
		% within Rose Bengal stain	0.0%	100.0%	100.0%
		% within Toluidine blue stain	0.0%	90.0%	23.7%
Total		Count	28	10	38
		% within Rose Bengal stain	73.7%	26.3%	100.0%
		% within Toluidine blue stain	100.0%	100.0%	100.0%

*Sensitivity = 100%
  Specificity = 90%
Positive predictive value = 96.6%
Negative predictive value = 100%

The chi square test revealed p value 33.01 (p>0.05) suggesting there was no significant difference between the two stains. (Table 4)

Table 4 Comparison of staining between Rose Bengal and Toluidine blue

Rose Bengal stain	Toluidine blue stain		TOTAL N (%)
	Positive staining	Negative staining	
Positive staining	28(96.6%))	1(3.4%)	29(100%)
Negative staining	0	9(100%)	9(100%)
TOTAL	28(73.7%)	10(26.3%)	38(100%)
CHI SQUARE VALUE: 33.01 P VALUE: 0.001			

Out of 21 biopsied cases 15 cases showed hyperkeratosis without dysplasia, 3 cases of hyperkeratosis with mild dysplasia, 2 hyperkeratosis with moderate-severe dysplasia and 1 SCC.

Discussion

Oral cancer is a public health problem which carries significant morbidity and mortality. The International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) stresses on a fact that effective cancer control and screening strategies can reduce a third of a predicted 15 million cancer cases in the future and helps more effectively managing another third of them. [5] Therefore the development of a technique for non-invasively detecting dysplastic changes or helping the clinician choose the appropriate site for biopsy is greatly necessary.

Toluidine blue has been established as a diagnostic adjunct in detecting oral lesions related to invasive carcinomas, carcinoma in situ or early asymptomatic oral carcinomas.[1] One meta-analysis of Rosenberg *et al.* previously published reported sensitivity ranged from 93.5% to 97.8% and the specificity ranged from 73.3% to 92.9%.[6] Zang et al reported that TB not only detects high-grade dysplasia but detects Oral premalignant lesions with minimal or no dysplasia with high-risk clinical and molecular attributes.[7] Hence, this study was undertaken to assess the efficacy of RB stain by comparing with TB in early detection of PMDs.

In our study mean age of patients ranged from 38.16+/-9.6. This was similar to Minati et al where the maximum percentage of patients (55.47%) were seen in the 3rd decade followed by the 4th and 5th decades.[8] Starzynska et al reported 76% of patients were over 40 years old and the average age was 58 years.[9]

In the present study except for one case the biopsy site was selected when both the diagnostic tools stained the same area. In comparison with TB, RB showed 100% Sensitivity (SN), 90% specificity (SP), 96.6% positive predictive value negative predictive value 100%. Du Ge Fi at al compared Rose Bengal with biopsy and reported 93.9 and 73.7%, sensitivity and specificity. Mittal at al reported 90% SP in comparison to biopsy.

Out of 21 biopsied cases, 18 were oral leukoplakia 2 lichen planus and 1 SCC. 15 cases showed hyperkeratosis without dysplasia, 3 cases of hyperkeratosis with mild dysplasia, 2 hyperkeratosis with moderate-severe dysplasia and 1 SCC. In one case RB was positive and TB was negative, but histologically no dysplasia was observed. This added up to 7 accurate diagnoses by Toluidine blue and 6 cases by Rose Bengal. But one case was inadequate to predict whether TB was superior to RB in detecting biopsy site. Out of 21 biopsied cases 5 of them had dysplasia and 3/5 of them had mild dysplasia. This was not similar to Du Ge Fi et al study where 5/128 cases of mild dysplasia were detected. [4] In the present study, 3 lesions demonstrated early OSMF like changes histologically but clinically appeared like leukoplakia. Since the study criteria were to assess the dysplasia only, they were considered as biopsy negative for statistical analysis. But all of these cases were followed up long term and one lesion showed remission over a period of one year. Du Ge Fi et al stated that it was clinically desirable to set lesions with normal clinical manifestation but with low-grade dysplasia as positive results

to detect these lesions as early as possible and follow up appropriately. If low-grade Dysplasia were to be included into the group of negative results (benign lesions), there would be more possibilities for these lesions to lose follow-up because they are often asymptomatic and neglected by patients.

An interesting observation made in the study was that hyperkeratotic areas took up less RB stain compared to the periphery of the lesion which was not the case with TB. This could have been due to decreased absorption of stains as there were increased keratotic areas or the lesion was less dysplastic. Since Toluidine blue is regarded as a nuclear stain, selective dye uptake can be due to more nucleic 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 canaliculi are much larger than the normal epithelium, consequently allowing increased penetration of dye. [10]

To compare the feasibility of RB application, following observations were made in the study. Since it was not used commonly it was difficult to obtain the RB. The initial rinse used was distilled water compared to acetic acid in TB which increased the patient compliance to RB compared to TB. The time taken for RB staining was more (4 min) than TB (1 min) which makes it difficult to utilize in large sample studies or surveys. In terms of response to stain 4 - 5 patients complained of mild stinging sensation when TB was applied but none of the patients had any objections to RB application.

Limitations Of The Present Study:

- The present study consisted of small sample size and only positively stained lesions were selected for biopsy hence, interpretation of the staining was limited to certain lesions only.
- Patients were stained with both RB and TB consecutively which might have interfered with the results of TB staining.
- A colorimetric guide was not used which limited our ability to comment on the association between increased intensity with the presence of increased dysplastic changes.
- In our study, we observed that along with lesion normal mucosa also took up pink stain though it was not intense. . The fact that we did not know which structure took up the stain in normal mucosa limited our knowledge about the positivity of staining.

Future Recommendations

- This was a primitive study in comparing the diagnostic tools like Rose Bengal and Toluidine blue further studies with large sample size is advisable.
- Since the present study includes only leukoplakia and lichen planus future studies could focus on other PMDs also. The staining pattern in field cancerization should also be assessed by staining the adjacent normal appearing mucosa in high risk patients.

- Since TB was most accepted chairside investigation for PMDs we compared it with RB stain. Biopsy was taken only when the lesions took up both RB and TB. In future all lesions should be biopsied irrespective of staining pattern of lesion
- Use of colorimetric guide in diagnosing the case and its association with histologic grading should be considered.

Conclusion

The present study sheds light on the possibility of using RB as an alternative to TB in clinical settings. The lesions stained with RB selected for biopsy sites revealed its ability to detect the earliest stages of dysplasia equivalent to that of TB. Patients gave a better response to RB application than TB application though it was more time consuming than TB. Hence with support from further studies it could be used as a simple and non-invasive technique alternative to TB in screening high risk patients with better patient compliance and no unwanted discomfort to patients.

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