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ORIGINAL RESEARCH

Rose Bengal staining – diagnostic aid for potentially malignant and malignant disorders: A pilot study

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ABSTRACT

Background: The word “Cancer” itself is sufficient to cause a fear in the minds of people. Early detection of oral potentially malignant and malignant disorders is still a diagnostic challenge for most of the clinicians. In the present study, we have evaluated the efficacy of Rose Bengal staining in the detection of oral premalignant and malignant lesions.

Aim: The aim of the study was to evaluate the efficacy of the Rose Bengal staining for the identification of dysplastic areas clinically, and then correlating the findings with the histological grading of dysplasia.

Methods and Materials: A total of 20 patients were selected for the study. Patients had been evaluated clinically and histopathologically along with the grading of dysplasia, and Rose Bengal stain was painted over the lesional mucosa with the patient’s consent. Incisional biopsies were taken from the stained area of the Rose Bengal dye, and studied histopathologically. Grading of dysplasia and intensity of the Rose Bengal staining were found to be correlated. Chi square test was performed and a statistical significance of \( P < 0.001 \) was observed. Statistical significance was defined as \( P < 0.001 \).

Results and Conclusion: Grading of dysplasia and the intensity of Rose Bengal staining were directly proportional to each other according to this study. \( P \) value was found to be significant. Thus Rose Bengal stain can be used as a diagnostic aid in the detection of oral potentially malignant and malignant disorders.

Key words: Malignant disorders, potentially malignant disorders, Rose Bengal stain

The word “Cancer” itself is sufficient to cause a fear in the minds of people. Emphasis has been placed on early detection and diagnosis of potentially malignant and malignant disorders. Adjuncts for detection of lesions include toluidine blue staining, exfoliative cytology, use of Vizilite and Velscope etc. As there are some drawbacks associated with each technique, the research for the ideal material that would help in the early detection of oral cancer is still going on. Rose Bengal stain (RB), the 4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiododervative of fluorescein, can stain the desquamated ocular epithelial cells.¹ As observed by Norn, with an exposure as brief as 1 second, Rose Bengal predominantly stains the cell membranes, an increasing the concentration or time of exposure produces predominant nuclear staining.² RB staining has been even used to delineate the extent of the corneal and conjunctival neoplasms.³ Therefore, such characteristics of RB stain have prompted the researches to test its potential for the detection of oral precancerous and malignant lesions.¹

According to the previous studies, lesions highly stained by RB have a higher likelihood to be oral squamous cell carcinoma (OSCC) or epithelial dysplasia (DP), than those showing a mild staining. Thus, RB staining might have the potency to be used as a diagnostic aid to detect oral precancerous or malignant lesions for clinicians.¹

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Diagnosis only by clinical examination may lead to misdiagnosis and therapeutic errors, therefore clinical and histopathological features should always be correlated to obtain the confirmatory diagnosis. Thus, the aim of the present study was to correlate the clinical and histopathological features of potentially malignant and malignant disorders to obtain a confirmatory diagnosis,
along with evaluation of the efficacy of Rose Bengal Stain in determining the grade of dysplasia in these lesions.\(^1\)

**MATERIALS AND METHODS**

This study was conducted in the Department of Oral and Maxillofacial Pathology, M. M. College of Dental Sciences and Research, Mullana. The study group encompassed a total of 20 cases. The criteria to include the patient in the present study were: (i) white lesions with oral tobacco or betelnut related habits, and (ii) lesions causing a suspicion of malignancy. All the patients were evaluated clinically and provisional diagnosis was obtained.

**Staining procedure**

- The patients were asked to rinse their mouth with distilled water to clean the lesions for 1 minute;
- 1% Rose Bengal solution was applied with a cotton tip for 2 minutes;
- Patients were asked to rinse their mouth for one minute with distilled water to remove excess RB solution;
- Oral examination of the location, size, morphology and surface characteristics of the sites stained was carried out, and the intensity of the staining was evaluated. After this, incisional biopsies were taken from the stained area of Rose Bengal dye under infiltration with adrenaline with the patient’s consent. After the fixation, processing and hematoxylin and eosin stain, slides were examined histopathologically and grading of dysplasia and intensity of Rose Bengal staining were correlated.

**Statistical analysis**

Chi square test was performed and a statistical significance of \(P<0.001\) was obtained. Statistical significance was defined as \(P<0.001\).

**RESULTS**

Out of the 20 cases, 10 cases were diagnosed as homogeneous leukoplakia, 7 cases as speckled leukoplakia, 1 case as verrucous carcinoma and 2 cases as carcinoma of buccal mucosa and floor of the mouth. Out of the 10 cases of diagnosed homogeneous leukoplakia, 8 cases were Rose Bengal staining positive [Figures 1 and 2] and 2 cases were negative [Figures 3 and 4]. In Figure 3 mild coloring of the lesion could have been because of remnants of stain due to inadequate rinsing of the lesion with distilled water after staining. Histopathologically, 9 cases showed mild dysplasia and 1 showed moderate dysplasia. 2 cases of homogeneous Leukoplakia, showing mild dysplasia, were false negative for Rose Bengal staining. Out of the 7 cases diagnosed as speckled leukoplakia, all cases were Rose Bengal staining positive [Figures 5 and 6]. Histopathologically, 1 case showed mild dysplasia, 5 showed moderate dysplasia and 1 showed severe dysplasia. The case diagnosed as verrucous carcinoma histopathologically was also positive for Rose Bengal staining. Clinically 2 cases diagnosed as carcinoma of buccal mucosa and floor of the mouth were positive for Rose Bengal staining clinically [Figures 7 and 8]. Further, these cases were histopathologically diagnosed as well differentiated squamous cell carcinoma [Table 1]. When the intensity of staining and grading of dysplasia were compared, it was observed that intensity of staining was more in high grade dysplasia.

**Efficacy of Rose Bengal staining method**

Sensitivity and diagnostic accuracy of RB staining method to detect dysplasia and carcinoma was found to be 90%. \(p\) value was found less than 0.001, which was significant.

**DISCUSSION**

Considering the increased incidence of oral cancer globally, it is crucial to find measures which will help in the early detection and diagnosis of this condition, thus helping in reducing the patient morbidity and mortality. This pilot study shed light on an effective diagnostic aid that can be used for oral potentially malignant and malignant disorders. Norn established the concept that Rose Bengal stains the cells, wherever there is poor protection of the surface epithelium by the preocular tear film. This concept has also been extended to the interpretation of other lesions, such as epithelial dendrites of herpes simplex and zoster, dysplasia or squamous metaplasia of conjunctival squamous neoplasms.\(^2\) Mucus or mucous layer may block the Rose Bengal uptake. A primary epithelial abnormality i.e. dysplasia, metaplasia, virus infected cells or other forms of epithelial keratitis, can render the inability of epithelium to interact with the mucous layer, thus allowing the Rose Bengal staining. False negative results could be due to late clinical expression of genetically induced changes in the cells or inability of the stain to penetrate the deeper layers of epithelium showing the dysplastic changes.

| Clinical diagnosis | Rose Bengal staining | Histopathological diagnosis |
|--------------------|----------------------|----------------------------|
|                    | No. of cases | + | - | Mild dysplasia | Moderate dysplasia | Severe dysplasia |
| Speckled leukoplakia | 7 | 7 | Nil | 1 | 5 | 1 |
| Homogeneous leukoplakia | 10 | 8 | 2 | 7+2 | 1 | 0 |
| Carcinoma of buccal mucosa | 2 | 2 | Nil | Well differentiated squamous cell carcinoma |
| Carcinoma of floor of mouth | 1 | 1 | Nil | Verrucous carcinoma |
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Figure 1: Mild staining with Rose Bengal of homogeneous leukoplakia in one of the patients in the study

Figure 2: Mild dysplasia

Figure 3: Negative staining with Rose Bengal of homogeneous leukoplakia in one of the patients in the study

Figure 4: Mild dysplasia

Figure 5: Positive intense staining with Rose Bengal of non-homogeneous leukoplakia in one of the patients in the study

Figure 6: Moderate dysplasia

Figure 7: Positive very intense staining with Rose Bengal of Squamous cell carcinoma in one of the patients in the study

Figure 8: Well differentiated SCC
Rose Bengal (RB) staining results also seemed to be more promising in detection of dysplasia in precancerous or clinically benign lesions, when compared with toluidine blue.\(^1\) Ge-fei-Du et al. (2007) concluded in his study that Rose Bengal staining may be better than toluidine blue staining.\(^1\) 1% Toluidine blue is an effective method of picking up malignant changes in premalignant lesions,\(^5\) and Rose Bengal stain can also be used effectively for the same purpose. In case of low grade dysplasia, there would be more possibilities for these lesions to loose out on follow-up as they are often asymptomatic and neglected by patients. In our study, even the cases of mild dysplasia were detected by Rose Bengal staining. Rose Bengal stain had also been used for the staining of cystic disorders of corneal epithelium\(^6\) as well as the diagnostic test for Sjogren syndrome in eyes.\(^7\) RB staining was even used to delineate the extent of corneal and conjunctival neoplasms.\(^8\) Rose Bengal (RB) disodium is a well-known intravenous diagnostic agent that undergoes rapid hepatic excretion in an unmetabolized form;\(^8,9\) however, in a new formulation it may also be used for chemoablation of localized tumours. It could also benefit patients with metastatic melanoma.\(^10\)

Further studies are required for better understanding the potential of Rose Bengal stain in detecting the oral potentially malignant and malignant disorders. A control group was not used in the study, as the research was done as an attempt to know about the feasibility of the rose Bengal stain in relation to oral potentially malignant and malignant disorders, and with the intention that even low grade dysplasia should not be ignored. In future, further research work can establish the Rose Bengal stain as a valuable diagnostic test in the detection of oral potentially malignant and malignant disorders.

In our study, sensitivity and diagnostic accuracy of RB staining method to detect dysplasia and carcinoma was found to be 90%. These results were comparable with the toluidine blue staining in the previous studies.

**CONCLUSION**

Rose Bengal staining can be used as a valuable diagnostic test in the detection of oral potentially malignant and malignant disorders.

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