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Case Report

Evaluating the Effectiveness of Toluidine Blue for Obtaining Safe Margins in Resection of Oral Squamous Cell Carcinoma

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Abstract

Aims: To evaluate the effectiveness of Toluidine Blue for obtaining safe margins in Oral Squamous Cell Carcinoma.

Materials and Methods: Intra-operatively irrigation of the lesion and its surrounding areas was done with Toluidine Blue solution for 20 seconds. Followed by irrigation with 1% acetic acid to remove all the mechanically retained stain. The unstained margins were demarcated using incision placed by no 15 BP blade. The lesion was resected with a safe margin of 1 cm away from the stained tissue. Neck Dissection was done according to the nodal status. The tumor along with the resected margins was sent for histo-pathological examination. Statistical analysis was performed to calculate specificity of the vital stain. The cross tabulation between epithelium of the stained and unstained margins was done and subjected to Chi- square test to calculate the significance.

Results: The Toluidine Blue vital stain has a sensitivity of 93.33%. Out of 15 cases, 2 patients recorded positive excision margins leading to recurrence at primary site; 1 patients recorded positive excision margins leading to recurrence at secondary site; 1 patient recorded free excision margins but had recurrence at secondary site; Remaining 11 patients recorded free excision margins and did not have recurrence.

Conclusion: Vital staining with Toluidine Blue is concluded to be specific in demarcating the dysplastic tissue adjacent to the carcinomatous lesion, which when excised along with the adjacent dysplastic tissue leads to a decrease in the recurrence in Oral Squamous Cell Carcinoma cases. Furthermore it is inexpensive, easily available and does not add significantly to the operating time. Moreover it provides a gross visualization of dysplasia surrounding the lesion especially in cases where in the margins are not well defined. Hence Toluidine Blue can be a useful and inexpensive adjunct to identify margins intra-operatively in the current scenario where intra-operative frozen sections are not available.

Keywords: Toluidine Blue; Squamous Cell Carcinoma

Introduction

Oral cancer is one of the common cancers in the Indian subcontinent due to rampant use of tobacco. Oral Cancer is the sixth most common cancer in the world. It accounts for about 4% of all cancers and 2% of cancer deaths worldwide [1,2].

It has been found that the presence of dysplasia at the surgical margin is a predictor of local recurrence. It also increases the chances of developing local recurrences by 5 times [3]. The difficulty of identifying a clear margin may be one of the most common reasons to have a wide range of safe margin. Leaving this unresected can result in local recurrence of second primary

tumors [4]. The need for complete resection of dysplastic tissues surrounding the primary lesion of OSCC has been emphasized [3]. To ensure this, it is imperative to know the microscopic extent of the tumor invasion which is not possible using conventional clinical examination methods. To ensure tumor removal surgeon excises a "safe" margin around the tumor [5]. Techniques to identify these dysplastic margins are still not clear and new techniques are still being researched [4].

Toluidine blue has been known for various medical applications since its discovery by William Henry Perkin in 1856. Toluidine blue has been extensively used as a vital stain for mucosal lesions and also has found applications in tissue sections to specifically stain certain components owing to its metachromatic property [6]. It also provides the surgical team with straight forward method for precisely identifying the advancing edge of premalignant lesions [7].

The present study was conducted to evaluate the effectiveness of Toluidine Blue for obtaining safe margins in Oral Squamous Cell Carcinoma.

Materials and Methods

Criteria for selection of subjects: Patients who were clinically and histopathologically diagnosed with squamous cell carcinoma of the oral region.

Inclusion criteria

Patients who were diagnosed with Oral squamous cell carcinoma with TNM staging of T1, T2, T3, N0, N1, N2 lesions were included in the present study.

Exclusion criteria

Patients who were diagnosed with Oral squamous cell carcinoma with TNM staging of

- T4 stage lesions,
- M1 OR M2 stage lesions
- Patients undergoing Chemotherapy or Radiotherapy,
- Patients who were diagnosed with Oral squamous cell carcinoma of the floor of the mouth were excluded from the study.

Study design

15 patients who were diagnosed histo-pathologically with Oral squamous cell carcinoma at the Al-Badar Dental college and Hospital, Gulbarga were taken up for study.

Investigations

- Routine blood investigations
- Contrast CT scan was done of the Maxilla, Mandible and the Neck. Axial, Coronal and Sagittal sections of the scan was then studied to know the extent of the lesion and lymph node involvement. OPG was also done to know the involvement of the bone.
- Chest X-ray and ECG were taken.
- USG Abdomen was done to rule out any organ metastasis.

Intraoperative after examination of lesion followed Photograph, irrigation of the oral cavity with saline was done to clean the area and then allow to dry. Dry area was then irrigated with Toluidine Blue solution for 20 seconds. Toluidine Blue being acidophilic metachromatic dye was taken up easily by the cancerous dysplastic tissue. Then the stained areas were irrigated with 1% acetic acid to remove all the mechanically retained stain. Stained margins were demarcated using incision placed by no 15 BP blade. The lesion was resected with a safe margin of 1 cm away from the stained tissue. Neck Dissection was done according to the nodal status. The tumor along with the resected margins was sent for histo-pathological examination. The stained epithelium was then examined for dysplastic changes. Standard histo-pathological analysis protocols were followed for the operated patients. Histo-pathological margins of the excised specimen was examined for presence or absence of invasion by lesion or dysplasia.

Followed by neck dissection reconstruction was carried out using different types of myocutaneous flaps and buccal pad of fat.

Six months follow-up study with post-operative histo-pathological analysis was performed.

The results obtained were subjected to statistical analysis. Statistical analysis was performed to calculate specificity of the vital stain. The cross tabulation between epithelium of the stained and unstained margins was done and subjected to Chi-Square test to calculate the significance.

Results

Buccal Mucosa emerged as the most common site of occurrence for Oral squamous cell carcinoma (27%), followed by Gingivo Buccal Sulcus (20%) and lateral aspect of tongue as the commonly occurring site (20%), maxilla involved (13%) one each at posterior & anterior maxilla, followed by retro-molar area(13%) and soft palate was the least site of occurrence for Oral squamous cell carcinoma (7%). In our study, out of the 15 cases operated for Oral squamous cell carcinoma, there were 11 cases with no recurrence, 2 cases with recurrence at Primary Site of Lesion and 2 cases with recurrence at Secondary Sites The chi square value for different surgical margins was found to be 0. The p value showed no statistically significant difference of staining between different surgical margins. The chi square value for different margins of the excised lesion was found to be 0.188. The p value showed no statistically significant difference of free and positive margins among different margins of the excised lesion.

The chi square value Between the Criteria of this study was found to be 2.19. The p value showed no statistically significant difference of Percentages Among the three Criteria (Intra-Operative Staining, Post-Operative Histo-Pathological Reports and Recurrence of The Disease).

The $\chi 2$ value for different margins of the excised lesion was found to be 0.188. The p value showed no statistically significant difference of free and positive margins among different margins of the excised lesion. Hence indicating a direct Co-Relation between Successful Intra-Operative Staining with Toluidine Blue Solution and Post-Operative Free Margin Histo-Pathological Reports. In our study the sensitivity of Toluidine Blue was found to be 0.933.Thus it shows that TB has a higher sensitivity when compared to other stains.

Discussion

Many factors are linked with the prognosis of treatment of Oral squamous cell carcinoma such as the tumor site, stage, thickness, histo-pathologic grade, microscopic invasion, cervical metastasis, number and anatomic level of positive nodes, presence and extent of extra-capsular spread. The status of resected margins has also been linked to prognosis [8]. Visual distinction between dysplasia and normal mucosa is based on differences between their color and texture. This makes visual clinical differentiation difficult. Studies have found a significant association between positive resection margins with development of loco-regional recurrence and also with a reduction in the disease free survival of the patient [9].

It was found that direct inspection of the lesion will always lead to varied results among examiners. Hence direct inspection is not reliable enough to delineate the range of dysplastic epithelium, especially by novice surgeons [10].

Vital stains have long been considered as a possible answer to this problem. The stains that have been tried are Toluidine blue, Lugol's iodine, Dental iodine glycerin 5% acetic acid, 0.4% indigo carmine and 0.5% Congo red [11-14].

The use of TB as a vital stain was first proposed by Richart to disclose dysplasia and carcinoma in situ of the uterine cervix. Neibel and Chomet and Shedd and co-workers were the first to report vital application of TB for the detection of premalignant and malignant lesions of the oral cavity [6].

In the present study, we used the Toluidine Blue to identify the squamous cell carcinoma margins intra-operatively to reduce errors in judgment made by clinical assessment. Toluidine Blue was chosen as the material of choice due to its low cost, wide availability and ease of use.

According to Umeda M., *et al*. 5 year disease specific survival rate was 93.8% with the use of lugol's Iodine [14], 100% with the use of Dental Iodine as assessed by Kurita H., *et al*. [10] while Toluidine Blue was found to be but without the use of any vital staining the survival rate declined to 75% [10].

The χ^2 value Between the Criteria of this study was found to be 2.19. The p value showed no statistically significant difference of Percentages among the Criteria.

Cases	Margins			
	Anterior	Posterior	Superior	Inferior
1	F M	F M	F M	F M
2	F M	F M	F M	F M
3	F M	F M	F M	F M
4	F M	F M	F M	F M
5	C i S	F M	F M	F M
6	I C	F M	F M	I C
7	F M	F M	F M	F M

8	F M	F M	F M	F M
9	M O D	F M	F M	F M
10	F M	I C	F M	F M
11	M O D	F M	M O D	F M
12	F M	F M	F M	F M
13	F M	F M	F M	F M
14	M O D	F M	F M	F M
15	I C	F M	F M	F M

Table 1: Post-Operative margins.

Keys: FM - Free Margins; MOD - Moderate Dysplasia; CIS - Carcinoma-*in-situ*; IC - Invasive Carcinoma.

Cases Without Any Recurrence	Cases With Recurrence At Primary Site	Cases With Recurrence At Secondary Site
11	02	02

Table 2: 6 months Follow-up for Recurrence.

	Anterior Margins		Posterior Margins		Superior Margins		Inferior Margins	
	Number	%	Number	%	Number	%	Number	%
Free margins	09	60.00	14	93.33	14	93.33	14	93.33
Carcinoma-in-situ	01	6.67	00	00	00	00	00	00
Invasive carcinoma	02	13.33	01	6.67	00	00	01	6.67
Moderate Dysplasia	03	20.00	00	00	01	6.67	00	00
Total	15	100	15	100	15	100	15	100
CHI SQUARE VALUE=12.5 (P = 0.188 > 0.05), Shows no significant difference of free and positive margins among different margins of the excised lesion.								

Table 3

Figure 1: Squamous Cell Carcinoma of Left Lateral Tongue.

Figure 2: Stained Dysplastic Tissue using Toluidine Blue.

Figure 3: Histopathological Procedure with Microscopical view of Margins.

Figure 4

Conclusion

Toluidine Blue is specific in demarcating the dysplastic tissue adjacent to carcinomatous lesion & increase the precision of the operating team in excising the dysplastic tissue surrounding the lesion leading to the decrease in recurrence at the primary site of the lesion. The stain was limited only to the upper layers of the epithelium hence depth evaluation by this stain is not possible. Sensitivity of Toluidine Blue was found to be 93.33%. The stain is constituted from materials which are easily available and inexpensive. Also, gives a gross visualization of dysplasia surrounding the lesion especially in cases where in the margins are not well defined. Hence Vital staining using Toluidine Blue is currently a useful and inexpensive adjunct to identify margins intra-operatively.

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