

Research Article

Evaluation of Safe Surgical Mucosal Margins with and without Intra-Operative Vital Staining with Toluidine Blue in Cases of Oral Squamous Cell Carcinoma – A Comparative Study

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Abstract

Purpose

To compare the safe surgical mucosal margin assessed intraoperatively with and without vital staining with Toluidine Blue (TB).

Method

A total of 240 mucosal margins obtained from 60 patients of histopathologically proven 'Oral Squamous Cell Carcinoma' (OSCC) between October 2016 to August 2018 were equally divided into two groups. In group I, vital staining of surgical margins with TB was done and in group II vital staining with TB was not performed (surgeons perception and frozen section biopsy was used to assess surgical mucosal margins).

Result

The results showed that group I had sensitivity 66.67% and specificity 100% with 100% PPV and 97.17% NPV. On the other hand, group II had 83.33% sensitivity, 100% specificity with 100% PPV and 99.13% NPV. The accuracy was 99.16% for both the groups.

Conclusion

Although there is no major advantage of use of TB in assessment of safe mucosal margin, it can be used as a useful substitute to conventional technique of assessment of surgical margin by surgeon's perception and frozen section in resource restrained centres where facility of frozen section is not available.

Introduction

The conventional treatment of OSCC comprised of wide local excision of the primary lesion with excision of normal tissue at margins in order to be at safe distance from tumor, with or without post-operative Radiotherapy or Chemotherapy. Tumor free margins is considered as the most important independent prognostic factor for overall survival, as incomplete removal of tumour would lead to local recurrence and may reduce the survival rate [1]. Often intra-operative methods of identification of tumor margins include visualization and palpation of resected margins by the operating surgeon. The decision regarding safe surgical margin by clinical palpation depends upon surgeons experience, expertise, accessibility of the tumor for palpation [2]. Sometimes there could occur some difficulties in clearly defining lesion's margins especially when there is a clinically dysplastic change or visible premalignant lesion in vicinity. Often the surgeons take clearance of 1 to 2 centimetres beyond the visible tumour margins considering tissue shrinkage, which could be inaccurate

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[3]. Moreover, there are chances of missing out microscopic spread of tumor by the surgeon's gross examination [4]. In literature various methods are mentioned for intraoperatively evaluation of safe surgical margin. These are: Frozen section biopsy, Touch Imprint Cytology, Micro endoscope and Optical Coherence Tomography. Frozen section biopsies are considered to be the standard screening modality for assessment of safe surgical margins intraoperatively. However, there are limitations to this technique. Expensive instruments, more space requirement and common technical difficulties making its availability to be limited [5]. Moreover, the frozen section facilities are not widely available in centres especially in developing countries and often the surgeons are compelled to take decisions arbitrarily. Vital staining of tissues could also be used as a modality to assess safe surgical margin [6–10]. Two techniques of vital staining are; intra-vital staining, i.e. in the living body (in-vivo) and supra-vital staining i.e. outside the body. Reichart in 1963 was the first to use TB for in-vivo staining of uterine cervical carcinoma in situ [10]. Other dyes that are commonly used in vital staining of tissues are Methylene blue, Lugol's Iodine solution, Acetic acid [11]. Out of these TB was reported to have more sensitivity and specificity in detecting dysplastic lesions when compared to acetic acid and lugol's iodine (other commonly used dyes) with higher positive and negative predictive values [12]. Dysplastic cells contain quantitatively more nucleic acids and there is loss of cohesion in the dysplastic epithelium. These features of dysplastic cells facilitate TB to penetrate through the epithelium and its retention in cancer cells, whereas normal mucosa fails to retain the dye[13]. As mentioned above, tumor resection by surgeons perception have disadvantage like arbitrarily taking clearance margins which may lead to inadequate resection. To overcome this limitation frozen section biopsy is considered as the gold standard for assessing the safe surgical margin intra-operatively. However, considering the expenses, availability, feasibility and technicality of frozen section, its use in margin assessment is limited. In the background of resource restrained centre, vital staining with TB could have a significant role in assessing surgical mucosal margins. Though these two aforementioned modalities have their own advantages and limitations, their efficacy to evaluate safe surgical mucosal margins need to be compared. Hence we proposed a study to assess the safe surgical mucosal margin by intra-operative vital staining with TB and without vital staining with TB where the margins were assessed by frozen section. Considering this, we hypothesized that the use of intra-operative vital staining with TB can be used as an adjunct to conventional technique of assessment of surgical margin by surgeon's perception and frozen section.

Methodology

The present prospective, observational study was conducted between October 2016 to August 2018 in Department of Oral & Maxillofacial Surgery, Sharad Pawar Dental College & Hospital and Acharya Vinoba

Bhave Rural Hospital Sawangi (Meghe), Wardha after obtaining approval for the study from the Institutional Ethical Committee of Datta Meghe Institute of Medical Sciences, Sawangi (M), Wardha, and Maharashtra. In the present study, 138 patients were screened and evaluated. Individuals with biopsy-proven oral squamous cell carcinoma with clinically respectable tumours and adequate mouth opening facilitating palpation of tumors were included in the study. While patients with ASA III/IV or with recurrent oral squamous cell carcinoma or who previously underwent surgery/chemotherapy/radiotherapy/chemo-radiotherapy and the histological variants of Squamous Cell Carcinoma - Papillary, Adenoid, Verrucous, Adenosquamous, Sarcomas, Basaloid and Spindle cell carcinomas, were excluded from the study. A total of 60 patients with histopathologically proven OSCC between October 2016 to August 2018 were included in the study depending upon inclusion and exclusion criterias. These were randomly divided using flip coin test into 2 groups: 1) Group I: With Vital staining with TB, 2) Group II: Without vital staining (surgeon's perception and frozen section biopsy was used to assess surgical mucosal margins).

Technique

The lesions were resected along with 1 -2 cms of clinically visible normal tissue. After excision of the lesion margins were excised from edge of the resected specimen. In group I, these margins were stained by TB. This was done by rinsing of the margins with normal saline for 30 seconds to remove debris. Then irrigation with 1% acetic acid for 30 seconds was done to remove ropey saliva. This was followed by application of 1% TB for 1 minute with cotton swab. Again, rinsing with 1% acetic acid was performed to remove mechanically retained stain. Finally the margins were again rinsed with normal saline. The interpretation was based on the colour; a dark blue (royal or navy) stain was considered positive, whereas, light blue staining and when no colour was observed, it was interpreted as negative stain. In Group II, vital staining with TB was not done. In this group the lesion was resected with clinically safe surgical margins (according to surgeon's perception). After resection of the lesion, margins were excised from the edges of the resected specimen, evaluated and sent for frozen section analysis. The results of frozen section were immediately reported to the surgical team, to aid in intraoperatively decision making. All the margins of group I and group II were finally sent for histopathologically analysis. A single senior pathologist examined these surgical margins. Only margins consisting of malignant tumor cells were considered as positive, while resection margins with dysplasia and with no malignant cells were considered as negative for malignancy. Statistical analysis was done by using descriptive and inferential statistics using chi square test, sensitivity, specificity, PPV (Positive Predictive Value), NPV (Negative Predictive Value) was done using SPSS 24.0 version (IBM corporation) and $p < 0.05$ was considered as level of significance.

Results

A total of 30 subjects fulfilling the inclusion criteria were included in each group - without vital staining group and with vital staining study group. The mean age of the without vital staining group was 52.63 9.20 and 48.40 12.36 of the with vital staining study group. There were 21 (70.0%) males and 9 (30.0%) females in without vital staining study group and there were 24 (80.0%) males and 6 (20.0%) females in with vital staining study group. Tumor differentiation and TNM classification of tumours are tabulated in

Table 1: Details of carcinoma of the study population

| Variables | Without Vital Staining (N = 30) | | | With Vital Staining (N = 30) | | |
|---------------|------------------------------------|----------|-------|---------------------------------|----------|-------|
| | Mean | S.D. | Range | Mean | S.D. | Range |
| Age | 52.63 | 9.20 | 35-65 | 50.50 | 12.28 | 25-72 |
| Sex | N | % | | N | % | |
| Male | 21 | 70.0 | | 24 | 80.0 | |
| Female | 9 | 30.0 | | 6 | 20.0 | |
| HD | | | | | | |
| MDSCC | 19 | 63.3 | | 19 | 63.3 | |
| WDSCC | 11 | 36.7 | | 7 | 23.3 | |
| PDSCC | 0 | 0.0 | | 3 | 10.0 | |
| SSC | 0 | 0.0 | | 1 | 3.4 | |
| Site | | | | | | |
| Alveolus | 3 | 10.0 | | 4 | 13.3 | |
| Buccal mucosa | 5 | 16.7 | | 4 | 13.3 | |
| GB sulcus | 16 | 53.3 | | 17 | 56.7 | |
| Labial mucosa | 3 | 10.0 | | 1 | 3.3 | |
| RMT | 1 | 3.3 | | 0 | 0.0 | |
| Tongue | 2 | 6.7 | | 4 | 13.4 | |
| T | | | | | | |
| 4a | 30 | 100.0 | | 30 | 100.0 | |
| N | | | | | | |
| 0 | 1 | 3.3 | | 0 | 0.0 | |
| 1 | 17 | 56.7 | | 15 | 50.0 | |
| 2a | 2 | 6.7 | | 1 | 5.0 | |
| 2b | 4 | 13.3 | | 2 | 6.7 | |
| 2c | 6 | 20.0 | | 13 | 43.3 | |
| M | | | | | | |
| 0 | 30 | 100.0 | | 30 | 100.0 | |
| Stage | | | | | | |
| Iva | 30 | 100.0 | | 30 | 100.0 | |

In the present study, out of 240 mucosal margins which were equally divided into two groups i.e. 120 in each group and evaluated, final histopathology examination revealed - 9 margins to be Positive for malignancy, and 231 margins to be Negative for malignancy. These results were considered as the 'Gold Standard' against which both vital staining with TB and without TB was compared. Out of 9 positive margins in histopathology report, 3 positive margins were found in group I and 6 in group II. In Group I only 2 out of 120 margins were stained positively with TB, however, upon histopathologically examination 3 out of these 120 margins were turned out to be positive for tumor infiltration implicating 2 true positive, 1 false negative, 117 true negative and none false positive.

Table 2: 2x2 contingency table for vital staining with TB (Group 1).

| 2x2 Contingency | | Histological Report | | |
|------------------------|----------|---------------------|----------|-------|
| | | Positive | Negative | |
| Vital staining with TB | Positive | n | 2 | 0 |
| | | % | 66.67 | 0.00 |
| | Negative | n | 1 | 117 |
| | | % | 33.34 | 100.0 |

Whereas, In Group II only 5 out of 120 margins were positive on frozen section report and upon histopathologically examination these 120 margins, 6 margins turned out to be positive for tumor infiltration implicating 5 true positive, 1 false negative, 114 true negative and none false positive.

Table 3: 2x2 contingency table for without vital staining with toluidine blue (surgeons perception) (Group2)

| 2x2 Contingency | | Histological Report | | |
|-----------------|----------|---------------------|----------|--------|
| | | Positive | Negative | |
| Frozen Report | Positive | n | 5 | 0 |
| | | % | 83.33 | 0.00 |
| | Negative | n | 1 | 114 |
| | | % | 16.67 | 100.00 |

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the both the groups were evaluated. The results showed that the group I had sensitivity 66.67% and specificity 100% with 100% PPV and 97.17% NPV (p=0.0001, significant). (Table 4) On the other hand, the group II had 83.33% sensitivity, 100% specificity with 100% PPV and 99.13% NPV (p=0.0001, significant). (Table 5) The accuracy was 99.16% for both the groups.

Table 4: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of vital staining with TB (Group 1).

| Vital staining with TB | % | 95% C.I. |
|------------------------|-------|----------------|
| True Positive | 66.67 | - |
| True Negative | 100.0 | - |
| Flase Positive | 0.00 | - |
| Flase Negative | 33.34 | - |
| Sensitivity | 66.67 | 9.43 to 99.16 |
| Specificity | 100.0 | 96.90 to 100.0 |
| PPV | 100.0 | - |
| NPV | 99.17 | 95.44 to 99.98 |
| Accuracy | 99.16 | - |

Table 5: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of without vital staining with toluidine blue (surgeons perception) (Group 2)

| Without vital staining with Toluidine Blue | % | 95% C.I. |
|--|-------|----------------|
| True Postive | 83.33 | - |
| True Negative | 100.0 | - |
| Flase Positve | 0.0 | - |
| Flase Negative | 16.67 | - |
| Sensitivity | 83.33 | 35.88 to 99.58 |
| Specificity | 100.0 | 96.82 to 100.0 |
| PPV | 100.0 | - |
| NPV | 99.13 | 95.01 to 99.85 |
| Accuracy | 99.16 | - |

Discussion

Outcome of surgical intervention of OSCC depends on plenty of prognostic factors. Most important of them is status of resection margins. Studies done on resection margins have shown that cases with a positive resection margin may have a higher incidence of loco regional recurrence [14]. Margins are usually considered positive only if it consists of malignant tumor cells. In addition, many authors found no significant difference in loco regional recurrence when resection margins with dysplasia were considered as positive margin [9,15]. Several studies had been done to reduce the incidence of positive surgical margin so as to minimize the rate of recurrence and thus mortality in cases of oral squamous cell carcinoma. In the routine scenario resection of tumor is based on the surgeon's perception to identify the tumor front and accordingly predict a safe surgical margin and these surgical margins are assessed by frozen section biopsy. Although surgeon's perception with various other modes of evaluation of surgical margins have their limitations in confirming complete resection of tumor with safe surgical margin [9]. Vital staining with TB is an established modality in the detection of the

oral malignant and pre-malignant lesion clinically. Since its discovery by William Henry Perkin in 1856, TB has been extensively used as a vital stain for dysplastic mucosal lesions. Drawback of TB is that it stains only three to four cell layers of mucosa [16]. So the extent of sub mucosal spread is difficult to appreciate. It has been shown to stain malignant lesions more effectively than the other vital stains [12] The sensitivity of TB in evaluating malignant and pre-malignant lesions ranges from 43.75% [17] to 97.29% [18]. While specificity ranges from 43.3% [19] to 88.89% [17]. Although, TB's ability to detect oral malignant lesions has been well documented in clinical settings, its use in intraoperatively identification of positive tumor margins has not been well established. Considering its ability to effectively stain malignant cells but not normal epithelium we postulated that vital staining with TB can be an effective screening modality for evaluating intra-operative adequate safe surgical mucosal margins in cases of oral squamous cell carcinoma. Early studies done by Nieble and Chomet [6] in 1964 and Strong [7] in 1968 reported that vital staining with TB effectively delineates intraepithelial neoplastic changes and could be used to evaluate the resected margins. In our study TB showed 66.67% sensitivity and 100% specificity with 100% PPV and 97.17% NPV. Similar studies have been reported to evaluate the efficacy of TB to assess safe surgical mucosal margin. Louis G. Portugal et al [8] in 1996 advocated use of TB at the time of resection to improve the ability to assess margin status. Montasir Junaid et al [10] in 2012 also supported the use of TB in assessing safe surgical margin. They demonstrated sensitivity and specificity of 100% and 97%, respectively and the diagnostic accuracy of 97.1% with a positive predictive value (PPV) of 27.2% and a negative predictive value (NPV) of 100%. C. J. Kerawala et al [9] in 2000 also advocated the usefulness of TB in identifying positive mucosal margins. However it does not show any advantage in identifying dysplasia in surgical margins. On the other hand the sensitivity and specificity of surgeon's perception aided with frozen section was found to be 83.33% and 100%, respectively, with 100% PPV and 99.13% NPV. With the comparable accuracy of 99.16% for both the modalities, this results show that vital staining with toluidne blue is equally effective in assessment of surgical margin as surgeons perception. In the present study the diagnostic accuracy of assessing a safe surgical margin by TB was similar to that of without TB application and hence it could be concluded that although there is no major advantage of use of TB in assessment of safe margin, it can be used as an useful substitute to conventional technique of assessment of surgical margin by surgeons perception and frozen section in resource restrained centres where facility of frozen section is not available.

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