

Efficacy of vital staining with iodine solution in reducing local recurrence in the resection of dysplastic or cancerous oral mucosa

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Abstract.

The purpose of this retrospective study was to assess the efficacy of vital staining with iodine solution in reducing local recurrence in the resection of dysplastic or cancerous oral mucosa.

The conventional group (a historical control group) underwent surgical resection of dysplastic or cancerous mucosa solely by direct inspection/palpation. The vital staining group did with the aid of vital staining with iodine solution.

Seven of 25 patients (28.0%) in the conventional group experienced recurrence of dysplastic or cancerous oral mucosa around the primary site, while no patient reported recurrence in the vital staining group (Fisher's exact probability test, $p < 0.01$).

Kaplan-Meier assessment showed that the 5-year primary-control rate was 100% in the vital staining group and 75% in the conventional group.

Although this retrospective study has some limitations, the results suggested the possible value of the vital staining with iodine solution in reducing the incidence of recurrence of dysplastic or cancerous epithelium at the same site. Further well-controlled study is mandatory.

Key words: vital staining, Lugol's iodine, squamous cell carcinoma, surgical margin,

local recurrence, oral dysplastic epithelium

Introduction

A number of studies have suggested that carcinoma cells and the degree of cellular atypia at resection margins may be significant in the development of local recurrence in the treatment oral malignant lesions.¹⁻³ It has been shown that failure to achieve a clear surgical margin results in increased risk of local recurrence and a subsequently reduced chance of survival.³⁻⁶

In oral cancer surgery, intraoperative margin assessment is usually performed using direct inspection and palpation and/or frozen section analysis; however, these methods have limitations in ensuring the complete resection of tumor and mucosal epithelium at a risk of malignancy. Vital staining with iodine solution has been employed intra-operatively with success in determining the extent and precise border of the cancerous/dysplastic epithelium, ensuring complete resection of the tumor at the mucosal surface.⁷⁻¹¹ The use of vital staining as an adjunct to determining resection margins for oral dysplastic/cancerous lesions may, theoretically, improve the margin control of oral malignant lesions. Therefore, the purpose of this retrospective study was to assess the efficacy of vital staining with iodine solution to control local recurrence in superficially spread possibly malignant or malignant lesion of oral mucosa.

Patients and Methods

Forty-eight patients were picked up from the cancer registry of the Department of Dentistry and Oral Surgery, Shinshu University Hospital from January 1986 to March 2005. These were patients who had a superficially spread dysplastic or cancerous oral mucosa and had undergone only surgical resection of oral mucosal lesion. Patients who had multiple origins, subepithelial infiltrating tumor (scirrhous carcinoma), a large tumor (T4), and/or tumor with neck lymph node metastasis were excluded. In addition, because normally keratinized epithelium does not usually retain iodine solution, patients with lesions on the gingiva or hard palate were not included.

In our department, the vital staining with iodine solution has been utilized since September 1990. Twenty-five consecutive patients, who were treated between January 1986 and August 1990, underwent surgical resection of dysplastic or cancerous mucosa solely by direct inspection/palpation (the conventional group, a historical control). Other 23 patients, who were treated between September 1990 and March 2005, underwent surgical removal of the oral epithelial lesion with the aid of vital staining with iodine solution (the vital staining group).

The sequence of vital staining with dental iodine glycerin (iodine 10 gm/100 ml) is

shown in Table 1. We prefer dental iodine glycerin because it has a higher concentration of iodine and more clearly demonstrates the contrast between normal and lesional mucosa rather than other iodine solutions, such as Lugol's iodine solution.¹² In the vital staining group, after clinical examination of the lesion, vital staining was performed for the lesion and surrounding mucosa. The iodine solution produced a brown-black stain on normal, nonkeratinized mucosa but not in lesional mucosa. Consequently, vital staining clearly demonstrated the intraepithelial extent of the lesion. Surgical resection of epithelial lesion was then carried out with more than a 5-mm border of normal tissues around the iodine non-stained area (Figure 1). On the other hand, in the conventional group, the area of lesional mucosa was decided only by direct inspection and palpation. The lesion was also removed with more than a 5-mm superficial surgical margin. In both groups, all surgical resections were carried out by trained and certificated oral surgeons.

All records were reviewed and the following data were collected for each patient: sex, age, primary site, maximal diameter of primary lesion, histological findings, histological surgical margin assessment, and outcome. All patients were basically followed up on a regular basis: every 2 weeks for the first year, every month for the second year, every 3 to 6 months for life. Their outcome as of 1 March, 2008 was recorded.

The time to recurrence of dysplastic or cancerous oral mucosa around the primary site (primary control rate) was compared between the two groups using the Kaplan-Meier actuarial method.¹³ Calculations was started at the time of the patient's first visit. Any presence of histopathologically proven premalignant or malignant lesions adjacent to the resected primary site were considered uncensored observations for the time to primary recurrence analysis. If patients underwent radiation therapy for oral cavity or systemic chemotherapy for second or metastatic lesions, the patients were then considered as censored. Analyses were performed using the StatView software package for Macintosh (SAS Institute, Inc., NC, USA). All p values < 0.05 were considered to indicate significance.

This study was approved by the Committee on medical Research of the Shinshu University.

Results

The clinical data of both groups are summarized in Table 2. Although the vital staining group tended to have more advanced histology (more invasive carcinoma) and larger lesions (larger maximum diameter), the differences were not statistically significant

(goodness test for fit for chi-square and Student's t-test, respectively). The follow-up period of the vital staining group was longer than that of the conventional group, but the difference was not significant (Mann-Whitney U-test). There were also no significant differences in other clinical characteristics.

In the period of this follow-up study, seven of 25 patients (28.0%) in the conventional group experienced recurrence around the primary site, while no patients reported recurrence in the other group. The higher prevalence of recurrence in the conventional group was statistically significant (Fisher's exact probability test, $p < 0.01$). Four patients with SCC experienced recurrence of SCC in mucosa adjacent to the primary site. Two patients with dysplastic epithelium and a patient with verrucous carcinoma experienced re-occurrence of dysplastic epithelium adjacent to the excised mucosa. Clinical and histopathological assessment of recurrent lesions revealed no possibility of recurrence from the deep surgical margin.

The results of Kaplan-Meier assessment of the primary control rate are shown in Figure 2. The primary-control estimate for patients in the vital staining group was 100% at 1 and 5 years and 91% at 1 year and 75% at 5 years in the conventional group (the log-rank test was not applied because no event happened in one arm).

Discussion

The purpose of this observation study was to assess the usefulness of vital staining with iodine solution for controlling local recurrence in oral cancer surgery. It must be stated that vital staining with iodine solution is only appropriate for dysplastic changes that appear on the mucosal surface. Vital staining with iodine solution has no value to detect subepithelially infiltrating carcinoma. In this study, therefore, the patients who had subepithelial infiltrating (scirrhous) tumor were not included. In addition, the patients who had multiple origins or wide field changes were also not included because adequate excision is impracticable in this group of patients. All patients underwent no treatments other than surgical resection for their oral mucosal lesions.

This observational study suggested that the vital staining with iodine solution might be useful in reducing the incidence of local recurrence in the resection of dysplastic or cancerous oral mucosa. In the results of this study, no local recurrence from the epithelium around the primary site was observed in the vital staining group, while a significant number of local recurrences were observed in the conventional group. It is well accepted that patients demonstrating a positive surgical margin (dysplastic/cancerous epithelium within the surgical margin) have a higher incidence of

local recurrence.³⁻⁶ Previously we showed that vital staining with iodine solution could delineate the precise area of the dysplastic epithelium.⁸ The dysplastic and cancerous epithelium was not stained with iodine solution, whereas the surrounding normal non-keratinized mucosa was stained brown-black. A 5-mm border of normal tissue peripheral to the iodine-positive area would be adequate for complete removal of the dysplastic epithelium.⁸ Complete resection of dysplastic epithelium ensured by vital staining with iodine solution might reduce the possibility of recurrence of dysplastic or cancerous oral mucosa at the same site.

In the results of this study, no local recurrence occurred in patients in whom the epithelium unstained with iodine was completely removed even with either histopathologically positive or negative margins. Yokoo et al.⁹ investigated the relationship between epithelial dysplasia unstained with iodine and the expression of proliferating cell nuclear antigen and/or the tumor suppressor gene (p53). They found that mild dysplastic epithelia stained with iodine may be in the category of normal epithelia, whereas both moderate and severe dysplasia unstained with iodine may be suggestive of malignant lesions. From these results, it was suggested that vital staining with iodine solution could detect epithelium with malignant potential.

Some surgeons feel that direct visualization of the oral mucosa is sensitive enough to identify suspicious lesions. In visual assessment, the distinction between dysplastic and normal epithelium is basically founded on differences in their color and texture; therefore, differentiation between dysplastic and the surrounding healthy epithelium is sometimes difficult if there is little difference. Previously, we assessed the inter-examiner reliability of examinations by either direct inspection or vital staining with iodine solution to detect a range of oral dysplastic lesions.¹⁴ An examination without vital staining showed a low interclass correlation coefficient of 0.62, as compared with 0.90 with vital staining. The results of examination by direct inspection varied widely among examiners; therefore, examination with only direct inspection was not reliable enough to delineate the range of dysplastic epithelium, especially if the surgeon was not very experienced.

Toluidine blue has been shown to enhance visualization of oral lesions and assist in identifying sites of increased risk of dysplastic/malignant change.²⁰ Previously, Kerawala et al.¹⁵ studied the efficacy of vital tissue staining with toluidine blue in the margin control of oral squamous cell carcinoma. They suggested that toluidine blue seems to be of no benefit for delineating positive resection margins and hence may be of little value for reducing the incidence of local recurrence. The ability of toluidine blue to stain

dysplastic tissue is believed to rely solely on quantitative differences in the amount of DNA and RNA.¹⁶ We think that the strict ability of toluidine blue staining to detect dysplastic lesions with quantity change of DNA and RNA was responsible for the low ability to detect positive surgical margins.

This retrospective study suggested the possible value of the vital staining with iodine solution in reducing the incidence of local recurrence in the resection of dysplastic or cancerous oral mucosa. Although the use of vital staining with iodine solution is limited to the mucosal surface and normally non-keratinized mucosa, it is of great value in determining the extent and precise borders of potentially malignant epithelium and thus, in reducing the incidence of local recurrence. This study has some limitations, including small number of samples, change in surgeons and techniques over the years, and study in single center. Further, well-controlled randomized study will be mandatory.

Conflict of interest statement

There are no financial or other relationships in regards to this submission that might lead to a conflict of interest.

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Figure Legends

Figure 1 Squamous cell carcinoma of the tongue. (A) Clinical appearance before the vital staining. (B) Appearance after application of iodine solution. Iodine solution produces a brown-black stain on normal, non-keratinized mucosa but not on lesional mucosa, clearly demonstrating the intraepithelial extent of the lesion. Surgical resection of epithelial lesion was carried out with more than a 5-mm border of normal tissues around the iodine non-stained area (a dotted line).

Figure 2 Primary control rates

Table 1

Sequence of dye application

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1. Clinical examination of lesion.
 2. Rinse with water and dry.
 3. Apply dental iodine glycerin with a cotton bud (10 to 20 seconds).

Formation of dental iodine glycerin:

Iodine	8 gm
Potassium iodine	8 gm
Zinc sulfate	1 gm
Glycerin	35 ml
Distilled water	65 ml

4. Wait for 1 to 2 minutes (and allow mouthwash with water).
 5. Interpret stain reaction.
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Table 2

Comparison of clinical characteristics between two groups.

	Vital staining group	Conventional group
Sex (Women : Men)	12:11	15:10
Age (Mean \pm SD)	62.7 \pm 13.7 years	58.9 \pm 17.7 years
Location		
Tongue	16	21
Lip or buccal mucosa	6	3
Floor of mouth	1	0
Oropharyngeal mucosa	0	1
Maximum diameter (horizontal)		
Mean (\pm SD)	27.6 \pm 14.1 mm	20.4 \pm 14.5 mm
Range	9–65 mm	3–60 mm
Pathological diagnosis		
Invasive SCC	13	9
Dysplastic epithelium	5	10
Carcinoma in situ	3	3
Verrucous carcinoma	2	3
Histological margin assessment of surgical specimen		
Positive for dysplastic epithelium	5	5
Negative	18	20
Follow-up period		
Median (IQR)	25 (9-50) months	18 (8-83) months

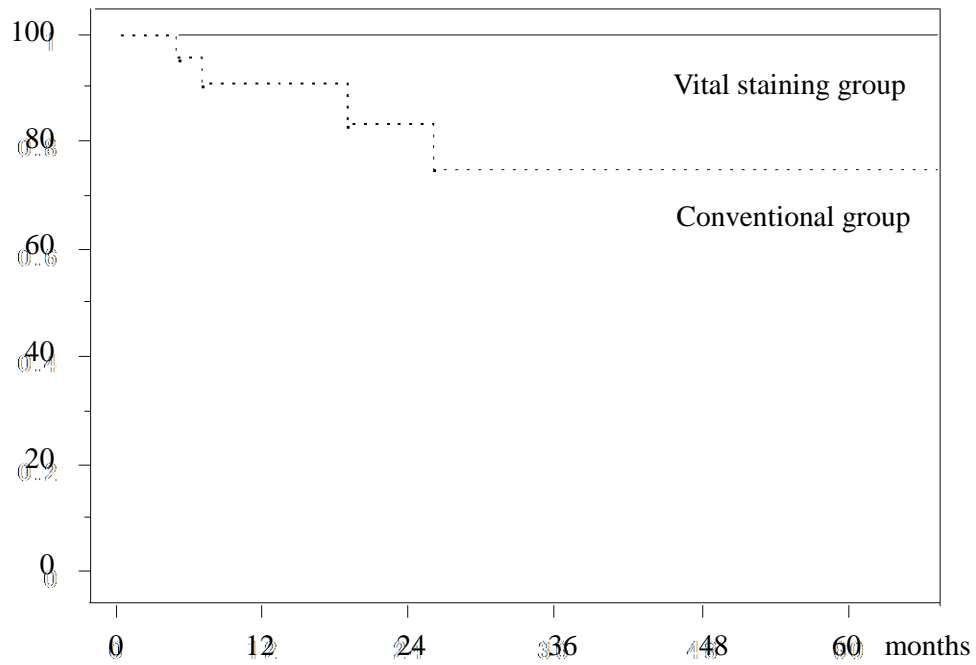
SD, standard deviation; SCC, squamous cell carcinoma; IQR, Inter-quartile range.

There were no any statistically significant differences in each clinical characteristic between the groups.

Figure 1



Figure 2



No. at risk

Vital staining group	(23)	16	15	10	7	5
Conventional group	(25)	18	11	8	8	7