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Combination of fluorescence visualization and iodine solution-guided surgery for local control of early tongue cancer

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Abstract. The control of enclosed oral epithelial dysplasia is important for the control of oral cancer. Fluorescence visualization and iodine solution are able to detect oral epithelial dysplasia and surrounding oral cancer. The purpose of this study was to clarify the effectiveness of combining fluorescence visualization and iodine solution-guided surgery for early tongue cancer. Participants comprised 264 patients with primary early tongue cancer who underwent surgery. The surgical margin was set at 10 mm outside the clinical tumour, and 5 mm outside the area of fluorescence visualization loss, and 5mm outside the iodine unstained area. The 5-year disease-free survival rate was 87.1% vs 76.1% ($P = 0.016$) and the 5-year local control rate was 98.6% vs 93.0% ($P = 0.008$) for combination-guided surgery when compared to conventional surgery. Positive margin rates were 0% for cancer, and 6.5% and 0% for low- and high-grade dysplasia, respectively, with combination-guided surgery ($P = 0.257$). Multivariate analysis revealed that combination-guided surgery (odds ratio 0.140, 95% confidence interval 0.045–0.437; $P < 0.001$) and intraoperative frozen section examination (odds ratio 0.302; 95% confidence interval 0.115–0.791; $P = 0.015$) were significantly associated with local control. The combination of fluorescence visualization and iodine solution are effective in selecting surgical margins for early tongue cancer.

Keywords: Fluorescence; Surgery; Cancer treatment protocol; Squamous cell carcinoma; Cancer of tongue.

Abbreviations: OSCC; oral squamous cell carcinoma; OEDoral epithelial dysplasia; IUiodine unstained; SCCsquamous cell carcinoma; FVLfluorescence visualization loss; LClocal control; UICCUnion for International Cancer Control; DOIpathological depth of invasion; OSoverall survival rate; DFSdisease-free survival rate; ORodds ratio; CIconfidence interval; CCLcollagen crosslink; NADHnicotinamide adenine dinucleotide; FVRfluorescence visualization retention; CKcytokeratin.

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Oral cancers and pharyngeal cancers account for about 4% of all cancer cases worldwide.[1] In Japan, these cancers accounted for about 2.4% of all cancer cases in 2021, with approximately 23,000 newly diagnosed cases and 7900 fatalities annually according to recent estimates.[2] Moreover, numbers of both newly diagnosed and fatal cases have increased five-fold in the last 40 years.[2]

One treatment for early oral squamous cell carcinoma (OSCC) is surgery.

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When attempting to excise early OSCC, macroscopically distinguishing the region of oral epithelial dysplasia (OED) from the normal-looking area surrounding the OSCC may be difficult for the surgeon. OED surrounding the OSCC is called field cancerization.[3] Leaving this OED unresected can result in local recurrence and regional and distant metastasis after resection.[2] The control of OED is therefore crucial for the control of early OSCC.

Iodine solution is a vital stain that has been reported as effective for detecting OED surrounding OSCC.[4] When iodine solution is used in tongue squamous cell carcinoma (SCC) and OED, an iodine unstained (IU) area is observed.[5] Fluorescence visualization is another non-invasive technique that has been gaining attention for the detection of OED.[6] OED and OSCC tissues show decreased autofluorescence in fluorescence visualization, appearing as a dark-brown area termed 'fluorescence visualization loss' (FVL).[6] Iodine solution[5] and fluorescence visualization[7] are reportedly useful methods in the treatment of tongue SCC, but it appears that no reports have described the utility of combining iodine solution and fluorescence visualization.

The aims of this study were to clarify the effectiveness of combined iodine solution and fluorescence visualization-guided surgery compared to conventional surgery with iodine solution for early tongue SCC, especially in terms of the local control (LC) rate.

Materials and methods

Patients

All patients with early primary tongue SCC (stage I or stage II) who underwent surgery between January 1997 and December 2019 at Tokyo Dental College, Chiba Hospital or the Chiba Dental Center were enrolled in this retrospective study. All patients were re-staged using the staging system of the eighth edition of the Union for International Cancer Control (UICC) TNM Classification of Malignant Tumours.[8] Conversation and swallowing function were evaluated using the Conversation Score, the Method of intake, Time of intake, and Food Score, and the Functional Outcome Swallowing Scale Score.[9]

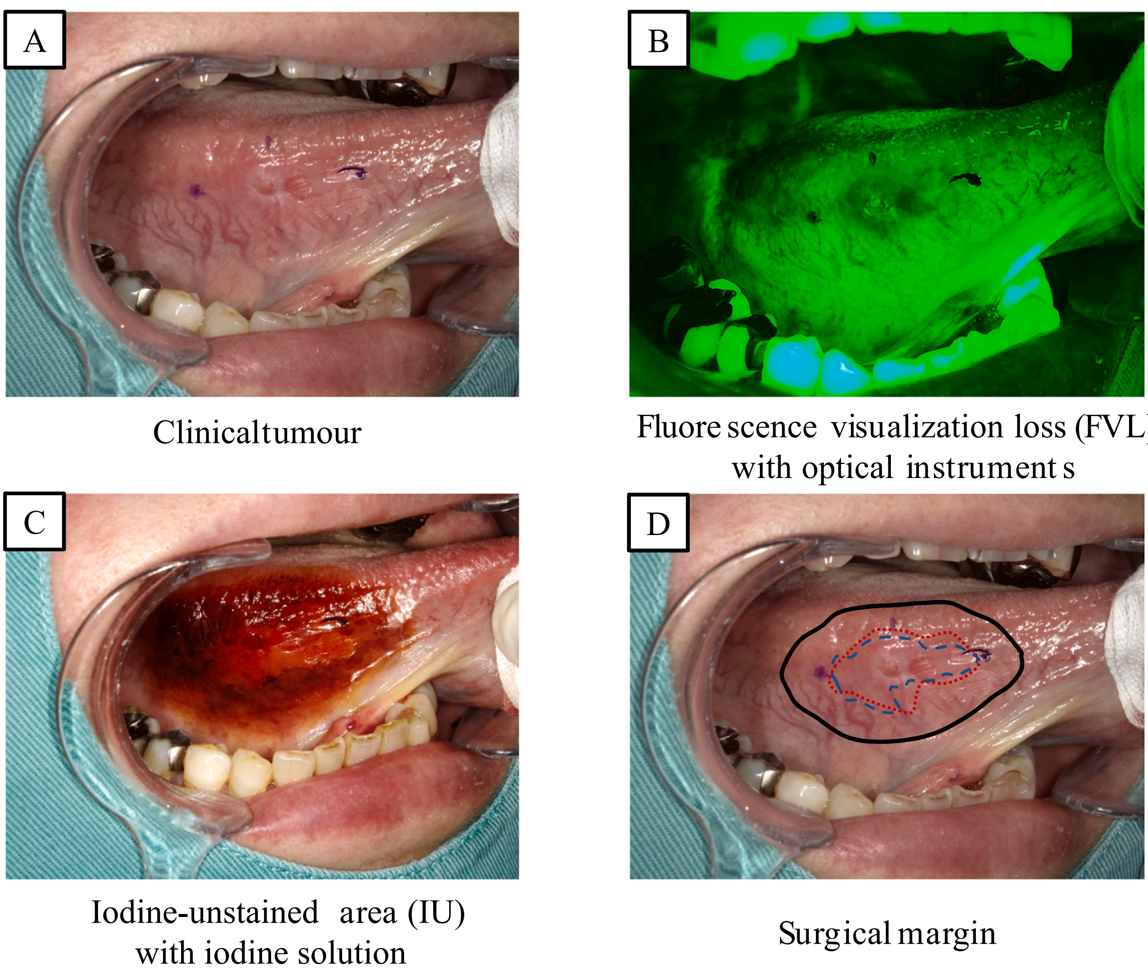

Fig. 1. Determination of the surgical margin; surgery for early tongue cancer. (A) Step 1 involves marking the macroscopic or palpated clinical extent of the tumour. (B) Step 2 involves marking the extent of fluorescence visualization loss (FVL) with the room lights turned off (FVL is shown by the dotted red line in image D). (C) Step 3 involves applying 3% iodine solution and observing the area not stained with iodine (iodine unstained area, IU) after 1 min (the IU area is shown by the dotted blue line in image D). (D) The surgical margin was determined using three criteria: 10 mm outside the clinical tumour, 5 mm outside the FVL, and 5 mm outside the IU area. The surgical margin is outlined with a black line and set as the maximum extent from each of these three criteria. In this case, FVL (dotted red line) and IU area (dotted blue line) are almost in agreement.

Inclusion criteria were a confirmed diagnosis of early tongue SCC according to the criteria of the eighth edition of the UICC TNM Classification of Malignant Tumours and a preoperative diagnosis of primary OSCC. Exclusion criteria were patients who rejected participation on opt-out and a history of other treatment for the head or neck before this surgery, such as radiotherapy or chemotherapy. The guidelines of the Japanese Society of Oral Oncology were used for macroscopic type, pathological depth of invasion (DOI), infiltrative growth pattern, and budding[10]; the UICC eighth edition criteria were used for histological grade,[8] and the binary system of the World Health Organization classification was used for OED.[11]

This study was approved by the Tokyo Dental College Ethics Committee (Authorization Number 740).

Method for selecting the surgical margin

Prior to surgery, the primary tumour was evaluated using intraoral ultrasonography, computed tomography, and/or magnetic resonance imaging. Fig. 1 shows the method used in the study hospitals to select the surgical margin. A combination of the area of the tumour determined clinically, IU area after the administration of iodine solution,[12,13] and FVL according to an optical instrument was used to select the surgical margin for early OSCC.[7,13]

At the time of surgery for early tongue SCC, three steps were applied. In step 1, the macroscopic or palpable clinical extent of the tumour was marked (Fig. 1A). In step 2, with the room lights turned off, the area of FVL was observed and marked (Fig. 1B; FVL shown with a dotted red line in Fig. 1D). In step 3, 3% iodine solution was applied, and the IU area was observed and marked after 1 min (Fig. 1C; IU area shown with a dotted blue line in Fig. 1D). The surgical margin was selected using criteria of 10 mm outside the clinical tumour, 5 mm outside the FVL, and 5 mm outside the IU area. The surgical margin was set as the maximum area delineated using these three margins (Fig. 1D).[13]

Excision was performed using a scalpel. Any mild bleeding after excision was controlled using electrocautery. The wound was closed with sutures or covered with a polyglycolic acid sheet. Intraoperative frozen section examination was performed after resection. If positive areas (including OED) remained on intraoperative frozen section examination, an additional resection was performed; this was repeated until negative results were confirmed.

Statistical analysis

The patients were divided into two categories based on how the surgical margin was set. Conventional surgery using iodine solution alone was performed between 1997 and 2009. Combination-guided surgery using both iodine solution and fluorescence visualization was performed between 2010 and 2019. The distribution of clinical and histopathological factors was tested by cross-tabulation and significance was tested using the χ^2 test and Kruskal–Wallis test. Positive rates for FVL and IU area in patients who underwent both fluorescence visualization and iodine solution examination during surgery were also examined.

The outcomes analysed included the overall survival rate (OS), disease-free survival rate (DFS), and LC rate. A survival analysis was performed using the Kaplan–Meier method and Cox regression analysis, and the stratified Mantel–Cox test was used to compare outcomes among the three groups. Multiple logistic regression was used to predict LC based on various clinical and pathological factors. The results of the multivariate analysis are presented as the odds ratio (OR) and 95% confidence interval (CI). IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Values of $P < 0.05$ were considered statistically significant.

Results

Patient and lesion characteristics

A total of 264 patients with primary early tongue SCC underwent surgery at Tokyo Dental College, Chiba Hospital or the Chiba Dental Center during the study period: 126 patients had conventional surgery and 138 patients had combination-guided surgery. No significant differences were found between the conventional and combination-guided surgery groups in terms of age, sex, histological grade, stage, DOI, perineural invasion, infiltrative growth pattern, budding, invasion into muscle, smoking, or drinking alcohol. In terms of the macroscopic type, 53.2% and 55.8% of patients in the conventional surgery and combination-guided surgery groups showed an endophytic type, and 33.3% and 20.3% showed the superficial spread type ($P = 0.018$). Blood vessel invasion was seen in 0.8% of the patients in the conventional surgery group and 8.0% of those in the combination-guided surgery group ($P = 0.005$). Lymphatic invasion was identified in 0% of patients who underwent conventional surgery and 5.1% of those who underwent combination-guided surgery ($P = 0.003$). Frequencies of comorbidities in the conventional and combination-guided surgery groups were 34.9% and 51.4%, respectively ($P = 0.007$) (Table 1). No adverse events related to fluorescence visualization were identified. Conversation and swallowing function scores did not differ significantly between the groups. The median duration of follow-up was 76.7 months (range 6.2–280.5 months).

Outcomes

For the overall cohort, 1-year, 3-year, and 5-year OS rates were 100%, 95.5%, and 94.5%, respectively. The 5-year OS was 93.4% with conventional surgery and 95.5% with combination-guided surgery ($P = 0.419$). For all patients, 1-year, 3-year, and 5-year DFS rates were 93.4%, 86.1%, and 82.0%, respectively. The 5-year DFS was 76.1% with conventional surgery and 87.1% with combination-guided surgery ($P = 0.016$). For all patients, 1-year, 3-year, and 5-year LC rates were 99.2%, 97.1%, and 94.2%, respectively. The 5-year LC rate was 93.0% with conventional surgery and 98.6% with combination-guided surgery ($P = 0.008$) (Fig. 2).

The intraoperative frozen section-positive rates (including OED) for conventional surgery and combination-guided surgery were 30.0% and 18.0%, respectively ($P = 0.249$). Regarding histopathology, the positive rate (including OED and SCC) was 10.3% with conventional surgery and 6.5% with combination-guided surgery ($P = 0.245$), and the SCC-positive rates were 0.8% and 0%, respectively ($P = 0.265$). Regarding OED alone, the percentage of cases showing low- and high-grade dysplasia were 8.7% and 0.8%, respectively, with conventional surgery and 6.5% and 0%, respectively, with combination-guided surgery ($P = 0.257$).

It was not possible to use iodine solution in five patients (1.9%) due to iodine allergy. Delineation rates of FVL and IU area in combination-guided surgery were 96.3% and 87.2%, respectively. The delineation rate tended to be slightly higher for FVL than for IU area, but the difference was not significant ($P = 0.724$). In this study, no cases were negative for iodine solution and/or fluorescence visualization. In terms of the positive area, FVL and IU area matched in 73.8% of cases. However, 13.5% of cases showed a FVL area greater than the IU area and 11.3% showed an IU area greater than the FVL area; 1.4% showed FVL and IU area were far.

Analysis of prognostic factors for local control

Table 2 shows the analysis of prognostic factors for LC. The factors identified as significant in the univariate analysis were budding ($P = 0.048$), intraoperative frozen section examination ($P = 0.002$), and combination-guided surgery ($P < 0.001$). Intraoperative frozen section examination (OR 0.302, 95% CI 0.115–0.791; $P = 0.015$) and combination-guided surgery (OR 0.140, 95% CI 0.045–0.437; $P < 0.001$) remained significant factors in the multivariate analysis.

Discussion

The concept of field cancerization is important for early OSCC control. Controlling OED is key to controlling early OSCC.[3] Detecting OED is therefore of particular importance. If the set margin for early OSCC surgery is too large, unnecessary scarring, deformation, and dysfunction (e.g., reduced range of motion, pronunciation disorders, and dysphagia) may result. However, if the set margin for early OSCC surgery is too small, residual cancer or OED tissue may cause local recurrence, lymph node metastasis, and distant metastasis.[1] Various approaches to complete resection of field alterations surrounding early OSCC have therefore been reported, including iodine solution[4,5,12,14] and fluorescence visualization.[6,13,15]

The application of iodine solution directly into the oral cavity is useful to identify tissue changes. This method is easy to use and observations can be made in real time. Iodine solution reportedly reacts with glycogen to produce a brown–black stain.[4,14] With dysplastic progression (as seen with low- or high-grade dysplasia, carcinoma in situ, and OSCC), the glycogen content of the granule cell layer will be decreased, therefore producing an IU area.[4] The delineation rate for IU area has been reported as 50.0–87.5% for OED and SCC,[4,14] and 71.0–85.0% for early tongue SCC.[4,16] In the region of the lesion border, low- and high-grade dysplasia have been observed in the IU area in 30.6% and 69.4% of cases, respectively[16].

In terms of the surgical margins determined using iodine solution, Umeda et al.[12] reported that, for surgery with a margin 5 mm outside the IU area in 93 patients with early tongue SCC, the positive margin rates for OED and SCC were 7.5% and 1.1%, respectively. Additional re-surgery was required in 6.4% of cases, but no local recurrence was identified. In the present study, the positive margin rates for OED+SCC and SCC were 10.3% and 0.8%, respectively, in those who underwent conventional surgery using iodine solution; 5-year LC was 93.0%.

Thus, iodine solution offers a good method for delineating OED. However, iodine solution has several limitations. First, the number of adaptation sites is limited. Adaptation sites for iodine solution are the movable mucosa, such as tongue and buccal mucosa, the oral floor, lip, and soft palate. Iodine solution is not applicable to keratinized mucosa, such as the upper and lower gingiva and hard palate.[4] Iodine solution has also been shown to be of no use for keratinized mucosa or inflammation tissue, as these tissues are less reactive to iodine.[14] Second, iodine solution has side effects and can be an irritant. Iodine solution is mildly invasive and is obviously contraindicated in patients who are allergic to iodine.[17] About 70% of patients report discomfort or pain with iodine solution.[17] Third, iodine solution produces a brown–black area that can render minute changes in tissues around the cancer difficult to identify.[13]

Table 1. Characteristics of the patients and lesions.

	Conventional surgery ($n = 126$)	Combination-guided surgery ($n = 138$)	P-value
Age (years), mean	60.0	62.8	0.051
Sex, male / female	72 / 54	68 / 70	0.201
Macroscopic type, superficial spread / exophytic / endophytic	42 / 17 / 67	28 / 33 / 77	0.018*
Histological grade, 1 / 2 / 3 / X	94 / 19 / 7 / 6	94 / 32 / 8 / 4	0.356
Stage, I / II	83 / 43	79 / 59	0.150
Depth of invasion (mm), mean	2.1	2.7	0.101
Blood vessel invasion, + / −	1 / 125	11 / 127	0.005*
Lymphatic invasion, + / −	0 / 126	7 / 131	0.003*
Perineural invasion, + / −	1 / 125	2 / 134	0.607
Infiltrative growth pattern, a / b / c	79 / 29 / 18	82 / 33 / 25	0.428
Budding, mean	2.1	2.7	0.076
Invasion into muscle, + / −	68 / 58	74 / 64	0.955
Inflammatory cell infiltration, + / −	108 / 18	124 / 14	0.303
Smoking, + / −	50 / 79	60 / 75	0.919
Drinking alcohol, + / −	77 / 49	72 / 66	0.314
Comorbidities, + / −	44 / 82	71 / 67	0.007*
Conversation score, mean	8.6	8.5	0.875
Method of intake, Time of intake, and Food score, mean	12.5	12.8	0.822
Functional Outcome Swallowing Scale score, mean	0.5	0.6	0.912

*Significant difference between the groups, $P < 0.05$.

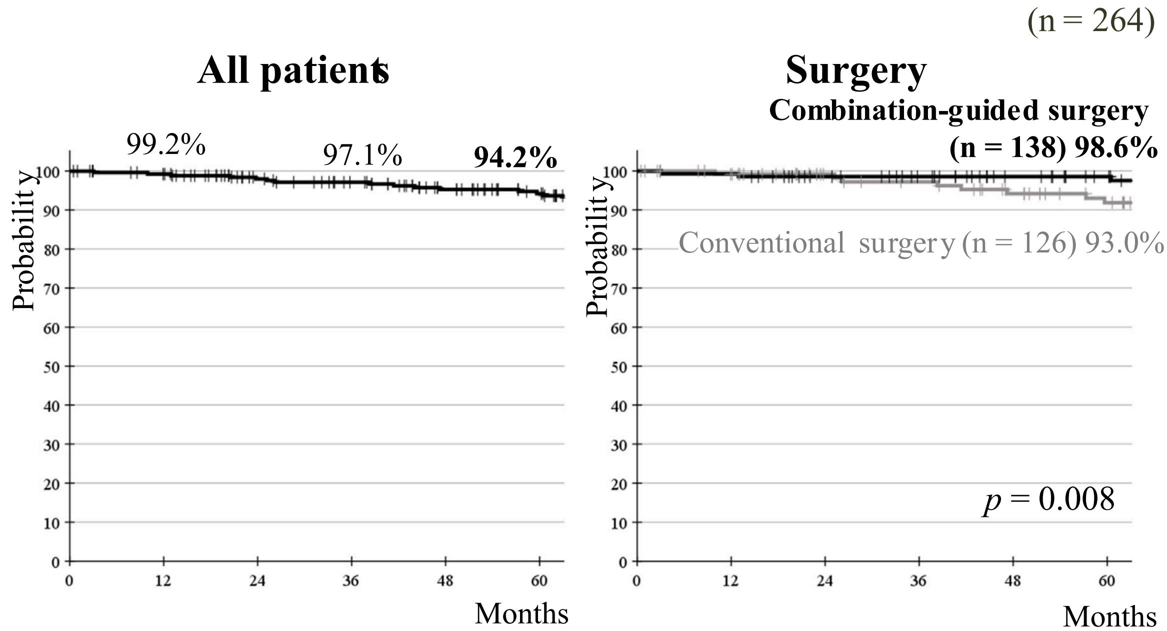

Fig. 2. Local control rate. In all patients, the 1-, 3-, and 5-year local control rates were 99.2%, 97.1%, and 94.2%, respectively. The 5-year local control rates with combination-guided surgery and conventional surgery were 98.6% and 93.0%, respectively ($P = 0.008$).

Fluorescence visualization uses a blue light (wavelength 400–500 nm) to illuminate collagen crosslink (CCL) and/or bio-coenzymes such as flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH) to produce autofluorescence.[18] A selective filter allows direct visualization of the apple-green autofluorescence that occurs in normal tissue as the area of fluorescence visualization retention (FVR). On the other hand, abnormal tissues such as OED and OSCC show decreased autofluorescence and appear as the dark-brown area of FVL.[6] This FVL is caused by absorption of a specific wavelength of blue light, such as with the breakdown of CCL, decreases in FAD or NADH, or angiogenesis.[6] CCL absorbs blue light and re-emits autofluorescent green light. The denaturation and destruction of CCL by dysplastic progression would thus cause FVL.[19] FAD and NADH are known to be involved in different types of intracellular energy metabolism, such as glycolysis, the tricarboxylic acid cycle, and the electron transport chain.[20] Dysplastic progression is known to enhance the form of anaerobic metabolism called the Warburg effect.[21] FAD and NADH are intermediate enzymes, and thus are consumed when anaerobic metabolism is enhanced. As a result, autofluorescence is decreased.[22] Furthermore, angiogenesis occurs due to cell proliferation,[23] and an inflammatory response is triggered by the immune response, resulting in FVL.[24]

Fluorescence visualization is easy to perform and non-invasive. In addition, the adaptation sites for fluorescence visualization are any sites of the oral mucosa. The great advantage of fluorescence visualization is the applicability to keratinized mucosa where iodine solution cannot be used.[15] Fluorescence

Table 2. Analysis of local control.

	Univariate analysis	Multivariate analysis		
	P-value	*P*-value	OR	95% CI
Age	0.717	–		
Sex	0.052	–		
Macroscopic type	0.810	–		
Histological grade	0.051	–		
Stage	0.149	–		
DOI	0.872	–		
Blood vessel invasion	0.280	–		
Lymphatic invasion	0.718	–		
Perineural invasion	0.748	–		
Infiltrative growth pattern	0.420	–		
Budding	0.048*	0.071	2.397	0.927, 6.200
Invasion of muscle layer	0.410	–		
Inflammatory cell infiltration	0.374	–		
Intraoperative frozen section	0.002*	0.015*	0.302	0.115, 0.791
Smoking	0.287	–		
Drinking alcohol	0.271	–		
Comorbidities	0.370	–		
Combination-guided surgery	< 0.001*	< 0.001*	0.140	0.045, 0.437

OR, odds ratio; 95% CI, 95% confidence interval; DOI, pathological depth of invasion;
*Significant, $P < 0.05$.

visualization thus makes up for the disadvantages of iodine solution. Fluorescence visualization involves a different mechanism of action from iodine solution, derived from CCL, FAD, NADH, and angiogenesis. Delineation rates based on FVL in the range of 77.8–98.0% have been reported for OED, carcinoma in situ, and OSCC,[6,25] and in the range of 87.1–100% for early tongue SCC.[7,13,16,25] In the region of the lesion border, low- and high-grade dysplasia have been observed in the area of FVL in 36.3% and 63.8% of cases, respectively.[16] It has also been reported that FVL shows a higher loss of heterozygosity than FVR.[15] Moreover, FVL reportedly shows a significantly lower cytokeratin (CK)13 expression rate than FVR, and a higher CK17 expression rate.[16] In other words, the area of FVL is considered to be an area with high cancer-forming ability.

In a study comparing fluorescence visualization and iodine solution, early tongue SCC showed a delineation rate of 100% on FVL and 72.5% on IU area.[16] FVL offered a slightly higher delineation rate than IU area.[16] In the present study, the delineation rates of FVL and IU area were found to be 96.3% and 87.2%, respectively. These delineation rates were not significantly different, but the delineation rate again tended to be higher for FVL than for IU area ($P = 0.724$). Iodine solution cannot be used in patients with an allergy to iodine, so fluorescence visualization is an effective alternative for those patients who are allergic to iodine.[17]

In terms of local recurrence, surgery with a margin of 10 mm outside the FVL showed a 3-year local recurrence rate of 6.5% in a study on 92 patients with early tongue SCC,[7] and 9.4% in 211 patients with early tongue SCC and carcinoma in situ, and high-grade dysplasia.[26] The background of combination-guided surgery was significantly worse than that of conventional surgery in this study.[10] However, the results of the present study showed that combination-guided surgery produced better outcomes in terms of DFS and LC compared to conventional surgery. In particular, the LC rate with combination-guided surgery was as high as 98.6%, and the impact on LC was substantial. As shown in the multivariate analysis, combination-guided surgery and intraoperative frozen section examination were prognostic factors for LC. The significance of intraoperative frozen section examination is very high, because changes in treatment policy can result from intraoperative frozen section examination. However, intraoperative frozen section is a special pathological diagnosis that requires high-level specimen preparation skills and diagnostic ability, and so cannot be performed at all facilities.[27] It is generally difficult to evaluate OED because the specimens for intraoperative frozen section are frozen specimens and the specimens are fragile. On the other hand, fluorescence visualization is simple and easy to use.

Sridharan et al.[28] reported that one factor for LC of early tongue SCC is distance to the closest margin. The reported OR of the margin clearance factor was 0.73. In the present study, the positive rate for SCC in histopathology was 0% and for OED was 6.5% in the patients who had combination-guided surgery. In addition, this OED with combination-guided surgery showed only low-grade dysplasia. Hence it is considered that the combination of fluorescence visualization and iodine solution facilitated the setting of appropriate margins for early tongue SCC. In the present study, the OR for combination-guided surgery was 0.14. By selecting an appropriate surgical margin, the intraoperative frozen section-positive rate and histopathology were decreased, and LC was further improved, resulting in a decrease in delayed neck lymph node metastasis and distant metastasis, leading to improvements in DFS. The rate of FVL delineation was excellent, but the matching rate with IU area with respect to the extent of the clinical tumour was 73.8%. These results suggest a difference in the mechanisms of action between iodine solution and fluorescence visualization. In other words, these promising results for guided surgery using iodine solution and fluorescence visualization were obtained by making use of differences in the respective mechanisms of action.

The negative side effects of this surgery caused by wide excisions are scarring, deformation, and dysfunction. In this study, conversation and swallowing function were evaluated, and no difference was found between the conventional surgery and combination-guided surgery groups. It is considered that the side effects of combination-guided surgery are not much different from those of the conventional surgery, suggesting the effectiveness of this method.

Several limitations of this study merit consideration. First, this study included a small number of patients and was retrospective in design. The OS rate may have been affected in other items. More rigorous prospective studies will be needed to confirm the results. In addition, both conventional surgery and combination-guided surgery performed well, so it may not be possible to show their effectiveness unless the number is further increased. Second, the methods used to evaluate iodine solution and fluorescence visualization were subjective, and more objective indicators may be necessary.[13] In the prospective study by Durham et al.,[26] fluorescence visualization-guided surgery did not show effectiveness. However, fluorescence visualization was determined subjectively, and the determination of FVL may have varied from evaluator to evaluator. The use of subjective and objective indicators is beginning to be examined in oral cancer screening.[5,25] Further prospective studies of OSCC screening and treatments for other oral subsites are therefore being planned.

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Competing interests

None.

Ethical approval

This study was approved by the Tokyo Dental College Ethics Committee (Authorization Number 740; date of approval November 15, 2016).

Patient consent

Consent was obtained from all participants in this study on opt-out.

References

1. Cancer Statistics. Cancer Information Service, National Cancer Center, Japan (Vital Statistics of Japan, Ministry of Health, Labour and Welfare).
2. Cancer Registry and Statistics. Cancer Information Service, National Cancer Center, Japan. ⟨https://ganjoho.jp/reg_stat/statistics/dl/index.html⟩ [Accessibility verified September 24, 2021].
3. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified

squamous epithelium. *Cancer* 1953;**6**:963–8.

4. Epstein JB, Scully C, Spinelli J. Toluidine blue and Lugol's iodine application in the assessment of oral malignant disease and lesions at risk of malignancy. *J Oral Pathol Med* 1992;**21**:160–3.
5. Yokoo K, Noma H, Inoue T, Hashimoto S, Shimono M. Cell proliferation and tumour suppressor gene expression in iodine unstained area surrounding oral squamous cell carcinoma. *Int J Oral Maxillofac Surg* 2004;**33**:75–83. https://doi.org/10.1054/ijom.2002.0457
6. Nagi R, Reddy-Kantharaj YB, Rakesh N, Janardhan-Reddy S, Sahuet S. Efficacy of light based detection systems for early detection of oral cancer and oral potentially malignant disorders: systematic review. *Med Oral Patol Oral Cir Bucal* 2016;**21**:447–55.
7. Poh CF, Zhang L, Anderson DW, Durham JS, Chen J, Berean KW, MacAulay CE, Rosin MP. Fluorescence visualization-guided surgery for early-stage oral cancer. *JAMA Otolaryngol Head Neck Surg* 2016;**142**:209–16.
8. Union for International Cancer Control (UICC). TNM classification of malignant tumours. Eighth edition New York: Wiley-Blackwell; 2017.
9. Japanese clinical practice guidelines for head and neck cancer 2018. Third edition. Tokyo: Kanehara Press, 2017.
10. Japanese Society of Oral Oncology and Japanese Society of Oral and Maxillofacial Surgeons. Clinical practice guidelines for oral cancer 2019. Third edition Tokyo: Kanehara Press; 2019.
11. El-Naggar AK, Chan JKC, Grandis JR, Tanaka T, Slootweg PJ. *WHO classification of head and neck tumours*. Fourth edition Lyon: IARC; 2017.
12. Umeda M, Shigeta T, Takahashi H, Minamikawa T, Komatsubara H, Oguni A, Shibuya Y, Komori T. Clinical evaluation of Lugol's iodine staining in the treatment of stage I–II squamous cell carcinoma of the tongue. *Int J Oral Maxillofac Surg* 2011;**40**:593–6.
13. Morikawa T, Bessho H, Nomura T, Kozakai K, Kosugi A, Shibahara T. Setting of the surgical margin using optical instrument for treatment of early tongue squamous cell carcinoma. *J Oral Maxillofac Surg Med Pathol* 2019;**31**:8–12. https://doi.org/10.1016/j.ajoms.2018.07.002
14. Takano M, Kakizawa T, Takasaki Y, Seta S, Noma H, Yajima Y, Nomura S. Clinical classification to indicate stage of oral precancerous lesions and early cancer with iodine and toluidine blue staining test. *Jpn J Head Neck Cancer* 2002;**28**:41–6. https://doi.org/10.5981/jjhnc1974.28.41. (In Japanese with English abstract).
15. Poh CF, Zhang L, Anderson DW, Durham JS, Williams PM, Priddy RW, Berean KW, Ng S, Tseng OL, MacAulay C, Rosin MP. Fluorescence visualization detection of field alterations in tumor margins of oral cancer patients. *Clin Cancer Res* 2006;**15**:6716–22.
16. Ikeda Y, Suzuki T, Saitou H, Ogane S, Hashimoto K, Takano N, Nomura T. Usefulness of fluorescence visualization-guided surgery for early-stage tongue squamous cell carcinoma compared to iodine vital staining. *Int J Clin Oncol* 2020;**25**:1604–11. https://doi.org/10.1007/s10147-020-01710-0
17. Elimairi I, Altay MA, Abdoun O, Elimairi A, Tozoglu S, Baur DA, Quereshy F. Clinical relevance of the utilization of vital Lugol's iodine staining in detection and diagnosis of oral cancer and dysplasia. *Clin Oral Investig* 2017;**21**:589–95.
18. Drezek R, Brookner C, Pavlova I, Boiko I, Malpica A, Lotan R, Follen M, Richards-Kortumetum R. Autofluorescence microscopy of fresh cervical-tissue sections reveals alterations in tissue biochemistry with dysplasia. *Photochem Photobiol* 2001;**73**:636–41.
19. Brookner CK, Follen M, Boiko I, Galvan J, Thomsen S, Malpica A, Suzuki S, Lotan R, Richards-Kortumet R. Autofluorescence patterns in short-term cultures of normal cervical tissue. *Photochem Photobiol* 2000;**71**:730–6.
20. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. *Annu Rev Phys Chem* 1996;**47**:555–606.
21. Harada K, Ferdous T, Harada T, Ueyama Y. Metformin in combination with 5-fluorouracil suppresses tumor growth by inhibiting the Warburg effect in human oral squamous cell carcinoma. *Int J Oncol* 2016;**49**:276–84.
22. Kosugi A, Kasahara M, Yang L, Nakamura-Takahashi A, Shibahara T, Mori T. Method for diagnosing neoplastic lesions by quantitative fluorescence value. *Sci Rep* 2019;**24**(9):7833 https://doi.org/10.1038/s41598-019-44287-z
23. Sekine R, Yakushiji T, Tanaka Y, Shibahara T. A study on the intrapapillary capillary loop detected by narrow band imaging system in early oral squamous cell carcinoma. *J Oral Maxillofac Surg Med Pathol* 2015;**27**:624–30. https://doi.org/10.1016/j.ajoms.2014.11.010
24. Marioni G, Staffieri A, Fasanaro E, Stramare R, Giacomelli L, Bernardi L, Val M, Stellini E, Filippis CD, Blandamura S. The role of angiogenin in pT1–T2 tongue carcinoma neo-angiogenesis and cell proliferation: an exploratory study. *J Oral Pathol Med* 2013;**42**:606–11.
25. Morikawa T, Kozakai A, Kosugi A, Bessho H, Shibahara T. Image processing analysis of oral cancer, oral potentially malignant disorders, and other oral diseases using optical instruments. *Int J Oral Maxillofac Surg* 2020;**49**:515–21. https://doi.org/10.1016/j.ijom.2019.08.016
26. Durham JS, Brasher P, Anderson DW, Yoo J, Hart R, Dort JC, Seikaly H, Kerr P, Rosin MP, Poh CF. Effect of fluorescence visualization-guided surgery on local recurrence of oral squamous cell carcinoma: a randomized clinical trial. *JAMA Otolaryngol Head Neck Surg* 2020;**146**:1149–55.
27. Byers RM, Bland KI, Luna M. The prognostic and therapeutic value of frozen section determinations in the surgical treatment of squamous cell carcinoma of head and neck. *Am J Surg* 1978;**136**:525–8.
28. Sridharan S, Thompson LDR, Purgina B, Sturgis CD, Shah AA, Burkey B, Tuluc M, Cognetti D, Xu B, Higgins K, Hernandez-Prera JC, Guerrero D, Bundele MM, Kim S, Duvvuri U, Ferris R, Gooding WE, Chiosea S. Early squamous cell carcinoma of the oral tongue with histologically benign lymph nodes: a model predicting local control and vetting of the eighth edition of the American Joint Committee on Cancer pathologic T stage. *Cancer* 2019;**15**:3198–207.

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