NONINVASIVE QUANTITATIVE ASSESSMENT OF ORAL TONGUE CANCER BY INTRAORAL ULTRASONOGRAPHY

Masashi Yamane, DDS,1 Junichi Ishii, DDS, PhD,1 Toshiyuki Izumo, DDS, PhD,2
Tohru Nagasawa,3 Teruo Amagasa, DDS, PhD1

1 Maxillofacial Surgery, Maxillofacial Reconstruction and Function, Division of Maxillofacial and Neck Reconstruction, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.
E-mail: m-yamane.mfs@tmd.ac.jp
2 Department of Pathology, Saitama Cancer Center, Saitama, Japan
3 Department of Healthcare Informatics, Takasaki University of Health and Welfare, Gunma, Japan

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Abstract: Background. To assess tissue characterization of oral tongue cancer and prediction of subclinical cervical lymph node metastasis, we investigated whether intraoral ultrasonography could be used in conjunction with a computer-aided diagnosis (CAD) system.

Methods. The study population comprised 109 patients with presurgical, clinical T1N0 or T2N0 oral tongue squamous cell carcinoma who underwent partial glossectomy. All the patients were examined by preoperative intraoral and postoperative ex vivo ultrasonography. To evaluate the ultrasonic images quantitatively, ultrasonographic parameters from tumor contour features were computed by using the proposed CAD system. The imaging results were correlated with histopathologic findings.

Results. Oral tongue cancer was clearly identified in all patients by intraoral ultrasonography. Ultrasonic images of oral tongue cancer reflected the histopathologic structures. Subclinical cervical lymph node metastasis was predicted by intraoral ultrasonography. In a logistic regression analysis using the proposed CAD system, the diagnostic sensitivity, specificity, and accuracy for prediction of subclinical lymph node metastasis were 87.2%, 84.3%, and 85.3%, respectively.

Conclusions. Intraoral ultrasonography in conjunction with the proposed CAD system allows tissue characterization and prediction of subclinical cervical lymph node metastasis.

Head and neck squamous cell carcinomas, including oral tongue cancer, account for approximately 5% of all carcinomas in industrialized countries; the worldwide incidence is 500,000 new cases annually.1 Despite significant advances in cancer therapy, the survival rate of patients with tongue squamous cell carcinoma has not changed in the last 30 years.2 Wide variety is observed in the external features and aggressiveness of tongue cancer during its natural history. Metastasis to the cervical lymph nodes is a crucial factor in the prognosis of tongue cancer. Therefore, there is an urgent need for methods to identify patients with clinically occult lymph node metastasis. At present, small nodal metastases (clinically occult disease) cannot be detected by contemporary diagnostic techniques. Many recent studies have identified tumor thickness (or depth of invasion)3–6
and histopathologic morphology at the invasive front as important prognostic factors of tongue cancer, particularly as predictors of cervical lymph node metastasis. In recent years, intraoral ultrasonography has been used to visualize and measure tumor thickness. However, the major focus of these studies was only to measure the tumor thickness. To date, the relationship between the ultrasonic images of oral tongue cancer and histopathologic features has not been elucidated. Accurate detection of tumor invasion of oral tongue cancer is essential for us to determine treatment for cervical lymph node micrometastasis. Ultrasonography is a highly operator-dependent technique. Therefore, some researchers have developed a computer-aided diagnosis (CAD) system. A CAD system can assist inexperienced operators to avoid misdiagnosis. However, an automatic CAD system for ultrasonic images is difficult to achieve, and to our knowledge, few satisfactory approaches are presently available.

By consensus, since malignant tumors infiltrate the surrounding tissues, the tumor contour on ultrasonic images shows an irregular shape and unsharp border. We hypothesized that the tumor outline on ultrasonic images may reflect the histopathologic morphology at the invasive front of oral tongue cancer. To evaluate the ultrasonic images quantitatively, we developed a novel CAD system that performed automatic computation of tumor contour features following segmentation of the tumor outline on ultrasonic images. Using intraoral ultrasonography along with the proposed CAD system, we prospectively evaluated the correlation between the ultrasonic images and histopathologic features of oral tongue cancer, and prediction of cervical lymph node metastasis. In the present study, we investigated whether intraoral ultrasonography can be used in conjunction with the proposed CAD system as a diagnostic tool for assessment of tissue characterization of oral tongue cancer, and prediction of subclinical cervical lymph node metastasis.

MATERIALS AND METHODS

Patients. Patients with squamous cell carcinoma of the oral tongue who underwent partial glossectomy between 1998 and 2002 at the Department of Maxillofacial Surgery, Tokyo Medical and Dental University Hospital (Tokyo, Japan) were enrolled in this study. Only patients with clinically-staged T1N0M0 or T2N0M0 disease who primarily underwent surgical treatment without prior radiotherapy or chemotherapy were enrolled. Cervical lymph node of patients was considered N0 when there was no palpable lymph node by physical examination and the size of the lymph node was <1 cm by both CT and ultrasonography without any area suggesting central necrosis or metastasis.

The study population comprised 109 patients (77 men, 32 women) with a mean age of 57 years (range, 21–90 years). All the patients had been followed up for at least 3 years or more. The preoperative clinical AJCC/UICC TNM stages were cT1N0M0 and cT2N0M0 in 41 and 68 patients, respectively.

The policy of treatment of oral tongue cancer was partial glossectomy for stage I and stage II cancer. Primary closure of the oral defect was possible for most of early-stage cancer. A large oral defect after excision of a large T2 tumor was reconstructed with a revascularized free radial forearm flap. Of the clinically N0 neck, the “wait and see” policy was employed. Elective radical or modified radical neck dissection was performed for N0 neck when the revascularized free radial forearm flap was used in the reconstruction of the oral defect. All the patients underwent partial glossectomy as the primary treatment, and 38 patients underwent elective neck dissections. All the patients provided written informed consent and completed the study.

Intraoral Ultrasonography. All the patients underwent an intraoral ultrasonographic examination before the operation. The transducer was covered with a disposable cover; sterile gel was applied, and the transducer was manipulated by placing it directly on the tumor surface. Scanning was performed without compression to avoid distortion of the tumor. To obtain the maximum cross-sectional area on the ultrasonic image, scanning was performed several times in the transverse plane of the tongue.

The ultrasound images were acquired using an Aloka SSD-630 ultrasound system (Aloka, Tokyo, Japan). A 10-MHz mechanical sector transducer (Aloka modified ASR-32 WU-10) was used. All data were recorded on a magneto-optical disk for the following offline data processing and computer analysis.

Ex Vivo Ultrasonography. After the partial glossectomy, the sample was pinned to a rubber backing. To match the ultrasonic images and histologic sections, the following procedures were performed. Two stainless steel pins were inserted at
both the ends of the central zone of the resected specimen, which is the thickest area of the tumor, to be used as ultrasonic reference points in the ex vivo studies. The specimen was then placed in a tank of degassed water with the tumor side of the tongue facing the ultrasonic transducer. The transducer was placed 5 to 10 mm from the specimen. The transducer was focused on the surface of the specimen near the reference points and displaced until specular reflections from the pins were found, and their center points were located.

Proposed Computer-Aided Diagnosis System for Ultrasonography. The proposed CAD system was composed of 2 components—automatic segmentation of tumor contour and computation of ultrasonographic parameters. This system was initially developed for breast nodules by a coauthor (TN). It can be freely downloaded from the Internet (http://www.takasaki-u.ac.jp/~nagasawa/index2.html) and runs on the Windows XP platform.

Automatic Segmentation of Tumor Contour. The segmentation method for tumor contour is shown in Figures 1A and 1B. First, a region of interest is manually selected from a region that extends beyond the lesion margins by 20 pixels in all directions. Second, a preprocessing step is applied for noise reduction using the median, mean, and low pass filters. Third, for image binarization, the threshold level is set using discriminant function analysis or variable threshold processing. Fourth, the edge of a contour is detected using a laplacian filter. Finally, the contour of the tumor area is automatically segmented by the edge-tracing method.

Computation of Ultrasonographic Parameters. The ultrasonographic parameters used were tumor thickness, irregularity of invasive front, and entropy of tumor contour. The measured and calculated parameters are described in Figure 1C and Table 1. The tumor thickness was measured vertically from the surface to the maximal thickness of the tumor, the accuracy was 0.1 mm. The irregularity of the invasive front was computed as follows:

\[ \text{Irregularity of invasive front} = \frac{E}{L} \]

where \( L \) is the linear distance of 60% of tumor width and \( E \) is the contour length of the interval. The value of irregularity of the invasive front increases with irregularity of its outline. To determine the irregularity of the tumor contour, entropy of the tumor contour was computed as follows: coordinates for the tumor contour as a complex plane were defined as \( Z_0, Z_1, \ldots, Z_n \). From the coordinate, displacement \( \delta_i = Z_{i+1} - Z_i \) was computed and normalized. The entropy of tumor contour was a probabilistic measure represented by the following equation:

\[ H = - \sum_k P_k \log_2 P_k \left( \sum_k P_k = 1 \right) \]

where \( P_k \) is the power spectrum computed by Fourier transformation of normalized displacement. When tumor contour becomes more irregular, the value of entropy increases, because the power spectrum extends.

### Table 1. Ultrasonographic parameters.

<table>
<thead>
<tr>
<th>Name</th>
<th>Definitions</th>
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<tbody>
<tr>
<td>Tumor thickness</td>
<td>Distance measured vertically from the surface to the maximal thickness of the tumor.</td>
</tr>
<tr>
<td>Irregularity of invasive front</td>
<td>( \frac{E}{L} )</td>
</tr>
<tr>
<td>L: linear distance that corresponds 60% of the tumor diameter.</td>
<td></td>
</tr>
<tr>
<td>E: contour length of invasive front corresponding section L.</td>
<td></td>
</tr>
<tr>
<td>Entropy of tumor contour</td>
<td>( H = - \sum_k P_k \log_2 P_k \left( \sum_k P_k = 1 \right) )</td>
</tr>
<tr>
<td>( P_k ): power spectrum of normalized displacement when tumor contour is considered to be a complex plane.</td>
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</table>
The above 2 steps were performed automatically, and the processing time was approximately 2 minutes.

**Histologic Study.** Following ex vivo ultrasonography, each specimen was fixed in 10% phosphate-buffered formalin. Then, each specimen was sectioned in the same plane as that of the reference points, which corresponds to the ex vivo ultrasonic images, and then embedded in paraffin. Subsequently, specimens were cut into 4-µm thick serial sections, and stained with hematoxylin–eosin for observation on histopathologic details and Azan-Mallory for detection of fibrous tissue. Immunohistochemical staining with cytokeratin AE1/AE3 (1:50, Dako Corporation, Carpinteria, CA) was performed for identification of cancer cells (nests), using dextran polymer conjugate 2-CA) was performed for identification of cancer cells (nests), using dextran polymer conjugate 2-

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Correlation between Ultrasonic Images and Histology. To evaluate the extent of tumor invasion and invasive front of tumor, each ex vivo ultrasonic image was matched and compared with its corresponding histologic section (see Figure 2). At the invasive front of the corresponding histologic section, tissue composition was examined by a pathologist (TI). Photomicrograph of the invasive front of tumor was captured using a charge coupled device (CCD) digital camera (DP11, Olympus, Tokyo, Japan) mounted on a standard microscope. The magnification was 25× and the visual field was 0.352 × 0.264 mm. For each specimen, at least 10 microscopic fields were captured. Using the public domain NIH Image software (version 1.61, developed at the U.S. National Institute of Health and available on internet at http://rsb.info.nih.gov/nihimage/download.html), histologic component was calculated as follows. The area percentage of tumor cells (nests) was calculated by selecting the area stained blue by the Azan-Mallory stain. The mean values of 10 fields were considered as the value of the selected zone.

**Statistical Analysis.** All values were expressed as the mean ± standard deviation (SD) when applicable. Correlation between ultrasonographic parameters (tumor thickness, irregularity of invasive front, and entropy) and histology (thickness and area percentage of fibrous tissue or tumor cells) was tested for significance using the Pearson's correlation coefficient. Differences in ultrasonographic parameters (irregularity or entropy) between modes of invasion were analyzed by 1-way analysis of variance (ANOVA) with Tukey's test. Univariate analysis was performed by Mann-Whitney U-test to assess the association of cervical lymph node metastasis with ultrasonographic parameters. All reported p values were calculated on the basis of 2-sided tests, and a p value less than .05 was considered statistically significant.

To identify the most important risk factor for cervical lymph node metastases, multivariate analysis was performed using the logistic-regression model. A forward stepwise procedure was used to select the independent variables that should have been included in the model to predict the dependent variables. A variable was progressively entered into the model if the significance level of its statistical score was less than 0.1. The risk score (S) for a patient is represented as S = B0 + B1 X1 + · · · + Bn Xn, where B0 is the constant and Bn is the regression coefficient of each independent variable Xn. The probability of metastasis is given as 1/(1 + e−S). A receiver operating characteristic (ROC) curve was used to identify the optimal cutoff score. The Hosmer-Lemeshow goodness-of-fit criterion was used to determine the adequacy of the final model. All statistical analyses were performed on a personal computer using the statistical package SPSS for Windows (Version 10.0, SPSS, Chicago, IL).

**RESULTS**

Comparison between Intraoral Ultrasonic Images and Histology. Tongue cancer was clearly identified in all patients by intraoral ultrasonography. The ultrasonic images of the tongue cancer showed a hypoechoic lesion when compared with the surrounding tongue tissue. Very thin lesions (as thin as 1 mm) could be detected, and high-
quality ultrasonic images detected the shape of the tumor invasion.

Tumor thickness was also easily measured by intraoral ultrasonography. Measurements on ultrasonic images yielded a thickness range of 1.0 to 24.0 mm. Scatter plots of data with regression lines were obtained. There was significant correlation between the measurements obtained by intraoral ultrasonography and those by histologic sections \( Y = 0.826, X - 0.253 \); \( Y \), tumor thickness on histologic section; \( X \), tumor thickness by intraoral ultrasonography; \( r = 0.985, p < .001 \).

The association between intraoral ultrasonographic parameters and the mode of invasion is shown in Figure 3. As the mode of invasion increased from grade 2 to grade 4D, the value of irregularity of invasive front and entropy tended to increase.

**Comparison Between Ex Vivo Ultrasonic Images and Corresponding Histology.** Histologic sections can be compared with the corresponding ex vivo ultrasonic images (Figure 2). The morphological features and extent of tumor invasion on the ultrasonic images correspond well with the histologic images of the same regions. On comparing, ultrasonography revealed additional details regarding the histologic structure of the tumors.

A significant correlation was detected between the ex vivo ultrasonographic parameters and histologic component. The value of irregularity of the invasive front correlated with the area percentage of fibrous tissue \( r = 0.581, p = .02 \) and the area percentage of tumor cells \( r = -0.646, p < .001 \).

**Univariate Analysis of Ultrasonographic Parameters in Cervical Lymph Node Metastases.** Cervical
lymph nodes were histologically positive in 39 of 109 patients (35.8%). Of the 38 patients who had elective neck dissections, micrometastasis was present in 6 patients (15.8%). Of the 71 patients on the wait and see policy, subsequent cervical lymph node metastasis was developed in 33 patients (47.1%), and they underwent therapeutic neck dissections.

The associations between intraoral ultrasonographic parameters and cervical lymph node metastases are presented in Table 2. Cervical lymph node metastasis correlated with entropy ($p < .001$), irregularity of invasive front ($p < .001$), and tumor thickness ($p < .001$).

**Multivariate Analysis of Ultrasonographic Parameters in Cervical Lymph Node Metastasis.** Forward stepwise logistic-regression analysis was performed on the parameters listed above. All 3 parameters were found to be independently related to cervical lymph node metastases (Table 3). The obtained regression model was as follows. The risk score ($S$) = $2.368 \times$ irregularity of invasive front + $1.169 \times$ entropy + $0.198 \times$ thickness − 11.262. From the ROC curve, a probability of 0.36 has the maximum specificity and sensitivity (see Figure 4). The results of classification by the model are shown in Table 4. With a cutoff score of 0.36, the sensitivity, specificity, and accuracy were 87.2% (34 of 39 patients), 84.3% (59 of 70 patients), and 85.3% (93 of 109 patients), respectively. Positive predictive value and negative predictive values were 75.6% (34 of 45 patients) and 92.2% (59 of 64 patients), respectively. The logistic regression model showed a good fit to the data using the Hosmer-Lemeshow goodness-of-fit ($\chi^2 = 12.91$, 8 degrees of freedom, $p = .115$).

**DISCUSSION**

The results of the present study indicate that intraoral ultrasonography with the proposed CAD system is a promising noninvasive quantitative method for assessment of tissue characterization of oral tongue cancer. The histopathologic features of the lymph nodes were histologically positive in 39 of 109 patients (35.8%). Of the 38 patients who had elective neck dissections, micrometastasis was

**FIGURE 3.** Box plots of the values of intraoral ultrasonographic parameters (irregularity of the invasive front or entropy), according to histologic grading of the mode of cancer invasion. The lower and upper limits of the boxes indicate the 25th and 75th percentiles; the horizontal lines and values within the boxes are the medians. Asterisks indicate significant difference ($p < .05$) between the 2 groups. As mode of invasion increased from grade 1, 2 to grade 4D, irregularity of invasive front and entropy tended to increase. Histologic grading of the mode of cancer invasion is as follows: 1, well-defined borderline; 2, cords, less marked border line; 3, group of cells, no distinct borderline; 4C, diffuse invasion of cord-like type; 4D, diffuse invasion of diffuse type.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lymph node metastases, mean ± SD</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregularity of invasive front</td>
<td>1.824 ± 0.34 1.419 ± 0.293</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Entropy</td>
<td>6.665 ± 0.658 5.789 ± 0.74</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tumor thickness</td>
<td>11.962 ± 5.546 6.639 ± 3.377</td>
<td>&lt;.001</td>
</tr>
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</table>
had previously been shown to correlate with nodal metastasis. We now show that ultrasonic images of oral tongue cancer correlated well with histopathologic features, and intraoral ultrasonography with the proposed CAD system can predict subclinical cervical lymph node metastasis with high sensitivity and specificity.

Oral tongue cancer could be clearly distinguished from the surrounding tissue by intraoral ultrasonography. This result was in agreement with those of Shintani et al and Helbig et al. However, unlike our study, these studies did not completely clarify the relationship between ultrasonic images and histologic sections. By focusing on the tumor-host borderline, ie, invasive front, we clarified the relationship, which has important clinical implications.

The invasive front is the most important area for prognostic determination of oral cancer. We hypothesized that the tumor outline of ultrasonic images may reflect the histopathologic morphology at the invasive front of oral tongue cancer. Therefore, we computed the irregularity of the invasive front and entropy as a parameter that represented the outline irregularity in ultrasonic images. This study has shown that tumors with a more diffuse invasion pattern were significantly associated with the high value of irregularity of invasive front and entropy. Why is invasive behavior correlated with the value of irregularity of invasive front and entropy? Ultrasonic images are obtained from radio frequency echo signals, which are created by reflections from interfaces between acoustically different regions and by incoherent scattering from tissue microstructures. Diffuse invasive carcinoma cells spread into the surrounding tissues and form microtumor nests. On the other hand, stromal cells surrounding the tumor nests show desmoplastic reaction. At the invasive front, microtumor nests can approximate the scattering region. Thus, the value of irregularity of the invasive front and entropy increase with diffuseness of invasive carcinoma because of incoherent scattering.

In addition, correlation was observed between the imaging results and histopathologic findings as well as ex vivo ultrasonography findings. In this study, the values of irregularity of the invasive front were positively correlated with the area percentage of fibrous tissues and inversely correlated with the area percentage of tumor nests. In the process of tumor invasion, tumor desmoplasia is induced in the matrix adjacent to the tumor cells, which show a more diffuse invasive pattern.

Oral tongue cancer has a propensity for occult lymph node metastases in clinically N0 patients. Micrometastasis (clinically occult disease) has been reported to occur in 30% to 40% of patients with early T1-2 tongue cancer. The presence of lymph node metastases directly affects the prognosis of the patients. However, these micrometa-

### Table 3. Results of logistic regression model predicting cervical lymph node metastases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regression coefficient</th>
<th>Odds ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregularity of invasive front</td>
<td>2.368</td>
<td>10.674 (1.443–78.941)</td>
<td>.02</td>
</tr>
<tr>
<td>Entropy</td>
<td>1.169</td>
<td>3.220 (1.002–10.350)</td>
<td>.047</td>
</tr>
<tr>
<td>Tumor thickness</td>
<td>0.198</td>
<td>1.218 (1.022–1.453)</td>
<td>.027</td>
</tr>
<tr>
<td>Constant</td>
<td>–11.262</td>
<td>&lt;.001</td>
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</tr>
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Abbreviation: CI, confidence interval.

FIGURE 4. Receiver operating characteristic (ROC) curve for the regression model in the classification of cervical lymph node metastasis. The area under the ROC curve is 0.889 ± 0.033. From the ROC curve, the cutoff score of 0.36 has the maximum sensitivity (87.2%) and specificity (84.3%).

### Table 4. Results of classification by regression model.

<table>
<thead>
<tr>
<th>Risk score</th>
<th>No. of patients by cervical lymph node metastases</th>
<th>Total no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &gt; .36</td>
<td>Positive 34, Negative 11</td>
<td>45</td>
</tr>
<tr>
<td>p ≤ .36</td>
<td>Positive 5, Negative 59</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>Positive 39, Negative 70</td>
<td>109</td>
</tr>
</tbody>
</table>
stases are not detectable by the best contemporary diagnostic technology. Depending on the delay in detection, these micrometastases might spread or become inoperable.

In our study, lymph node metastases could be accurately diagnosed by the proposed CAD system and the forward stepwise multiple logistic regression model. Accrued evidence indicates that our model yielded a markedly high sensitivity of 87.2%, specificity of 84.3%, and accuracy of 85.3%. Many studies have been conducted for the prognostication of lymph node metastases. However, their accuracy ranged from 59.3% to 77.2%, which is lower than our values. Furthermore, most of these studies used invasive examination.

Moreover, the significant finding in this study is a high negative predictive value of 92.2%. A high negative predictive value could potentially help avert elective neck dissection. The optimum management of the N0 neck in patients with oral tongue cancer remains a controversial issue. Several investigators have suggested that elective neck dissection may improve the treatment results in patients with oral tongue cancer. However, about 70% of the patients may result in unnecessary aggressive treatment. Our results could be used to redefine the criteria for the appropriate use of elective neck dissection and other therapeutic interventions noninvasively.

However, our study also has limitations. First, our study population was small, and these results should be considered preliminary. We recommend a multicenter validation study. Second, the ultrasonic information was obtained only from 2 perpendicular planes through the tumor and not from the entire 3-dimensional tumor structure, which is analyzed histologically. The development of real-time 3-dimensional imaging is necessary.

In conclusion, intraoral ultrasonography with the proposed CAD system allows tissue characterization and prediction of subclinical cervical lymph node metastasis of oral tongue cancer. We consider that further exploration of the proposed CAD system will be useful for predicting malignancy and prognosis not only for tongue cancer but also for a wide range of other malignancies.

REFERENCES