

学位論文の要旨

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学位論文名 : Applicability of Preoperative Nuclear Morphometry to Evaluating Risk for Cervical Lymph Node Metastasis in Oral Squamous Cell Carcinoma

発表雑誌名 : PLoS One
(巻, 初頁~終頁, 年) : (9(12):e116452. 2014.)

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論文内容の要旨

INTRODUCTION

We previously reported the utility of preoperative nuclear morphometry for evaluating risk for cervical lymph node metastases in tongue squamous cell carcinoma. The risk for lymph node metastasis in oral squamous cell carcinoma, however, is known to differ depending on the anatomical site of the primary tumor, such as the tongue, gingiva, mouth floor, and buccal mucosa. In this study, we evaluated the applicability of this morphometric technique to evaluating the risk for cervical lymph node metastasis in oral squamous cell carcinoma.

MATERIALS AND METHODS

Data collection

Data were retrospectively collected for patients who were histopathologically diagnosed with OSCC and underwent surgical management including neck dissection at the Department of Oral and Maxillofacial Surgery, Nagasaki University Medical and Dental Hospital between January 1986 and January 2001 and the Department of Oral and Maxillofacial Surgery, Shimane University Faculty of Medicine between 1981 and 2012. Recurrent cases were excluded.

Biopsy specimens and pathologic nodal classification

Biopsy was performed in all patients preoperatively and/or prior to neoadjuvant therapy. The biopsy specimens were fixed with 10% neutral buffered formalin for 24 h and were processed for routine paraffin embedded sections, then stained with hematoxylin and eosin.

All the lymph nodes dissected from the biopsy specimens were examined for pN status and level of the metastatic lymph nodes. Cervical lymph node level was determined based on the cervical lymph node metastatic guide.

Image analysis and nuclear parameter measurements

Images of each section were stored using a standard light microscope (using x10 objective lens) connected to a computerized digital camera. The image data were analyzed by Mac Scope software (Mitani Co., Fukui, Japan) to estimate the various quantitative nuclear features (at least 100 nuclei per case). Nuclear margins were digitally marked under high power view on the computer screen to ensure measurement accuracy.

Mean (standard deviation) values of the nuclear area and perimeter were calculated from counts of the pixels capturing the nuclei and their edges. The nuclear circular rate and aspect ratio were automatically calculated to determine variations in shape; briefly, in a round circle, the circular rate and aspect ratio values correspond to 1: if the object is elliptical, the circular rate is <1 and the aspect ratio is >1. NACV was calculated to express variations in size in individual cases. The study protocol was approved by the Ethics Committee of Shimane University Hospital (Approval No.: 1286).

Statistical analysis

To examine differences in patient characteristics between pN-positive and pN-negative patients, we performed *t*-tests for continuous variables. *p* values less than 0.05 were considered statistically significant.

Logistic regression analysis was performed to identify the risk factors for node-positive status. Odds ratios and confidence intervals (based on the Wald test) were also calculated. Candidate risk factors with *p* values less than 0.1 on the Wald test were selected. From among them, risk factors were determined by variable selection using Akaike's Information Criterion. The optimal cutoff values for measurements were obtained with the minimum *p* values from the *t*-tests. All analyses were performed using SAS version 9.3 software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Eighty-eight cases of squamous cell carcinoma (52 of the tongue, 25 of the gingiva, 4 of the buccal mucosa, and 7 of the mouth floor) were included: 46 with positive node classification and 42 with negative node classification. Nuclear area and perimeter were significantly larger in node-positive cases than in node-negative cases; however, there were no significant differences in circular rate, aspect ratio, or NACV. We derived two risk models based on the results of multivariate analysis: Model 1, which identified age and mean nuclear area and Model 2, which identified age and mean nuclear perimeter. It should be noted that primary tumor site was not associated the pN-positive status. There were no significant differences in pathological nodal status by aspect ratio, NACV, or primary tumor site.

A simple and reliable method for evaluating the preoperative risk for lymph node metastasis would be indispensable in routine clinical practice, and our approach requires no special equipment or staining technique. Several papers discuss that nuclear shape is a critical factor in the characterization of many neoplastic and non-neoplastic proliferations, and irregularity of the nuclear shape is one of the morphological characteristics commonly used to determine the type or degree of neoplastic transformation. Size and contour irregularities of the nuclei are important features in the grading of OSCC. Furthermore, it was reported that the nuclear size was larger in proportion to the grade of malignancy.

On the whole, among the quantitative morphometric parameters of the nuclei analyzed in this study, the results suggest that malignant nuclei become aspherical, which would be consistent with previous reports. Regarding the patient's age, however, it has been reported that low age (<42 years) was associated with the development of cervical lymph node metastasis within a short time frame (≤ 50 days). In the present study, age < 65 years was suggested to be a risk factor for cervical lymph node metastasis in OSCC.

Future studies also need to address the applicability of clinical and biological marker analyses to accurately evaluate metastatic potential in OSCC preoperatively. Recently, the nuclear factor kappa B was reported to be a key protein in multi-step carcinogenesis, lymph node metastasis, and prognosis of oral, head, and neck squamous cell carcinoma. The expression of a combination of nuclear factor kappa B or other markers should be examined further.

CONCLUSION

Our method of preoperative nuclear morphometry may contribute valuable information to evaluations of the risk for lymph node metastasis in oral squamous cell carcinoma.