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Implications of morphologic patterns of intraepithelial microvasculature observed by narrow-band imaging system in cases of oral squamous cell carcinoma

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SUMMARY

Purpose: To investigate the intraepithelial microvascular morphology of oral squamous cell carcinoma (OSCC) by using narrow-band imaging (NBI) and analyze whether the intraepithelial papillary capillary loop (IPCL) patterns correlate with infiltration depth and disease severity in OSCC.
Methods: The clinicopathologic data, morphology of vascular architecture as observed by NBI, and histopathology of patients with OSCC were retrospectively reviewed and analyzed.
Results: A total of 80 patients, including 73 males and 7 females with an average age of 54.18 ± 12.23 years, were enrolled. Three patterns of intraepithelial microvasculature were revealed by NBI and differences in these three patterns were significant with regard to pathologic T-classification ($p < 0.0001$), N-classification ($p = 0.00022$), TNM stage ($p < 0.0001$), lymphovascular invasion ($p < 0.0001$), perineural invasion ($p = 0.000299$), depth of tumor infiltration ($p < 0.0001$), and tumor differentiation ($p < 0.0001$). A cut-off point of tumor infiltration of 10.012 mm was best predicted for the destructive pattern of IPCL (sensitivity = 100%, specificity = 90.0%).
Conclusions: Three different patterns of IPCL, showing step-wise increased severity according to pathologic parameters, were observed by NBI in cases of OSCC. The pattern indicating IPCL destruction with angiogenesis was associated with more advanced disease stage.

Introduction

Narrowband imaging (NBI) is a novel, noninvasive optical technique that enables visualization of the mucosa surface and the capillary pattern of the superficial layer by using narrow-bandwidth filters in a sequential red–green–blue illumination system.[1,2] The central wavelengths of each band are 415 nm and 540 nm. The narrowband blue light, which has a short wavelength (415 nm), penetrates the mucosa and highlights the intraepithelial microvasculature.[3] Endoscopy with NBI system provides images with fine capillary patterns, allows detailed observation of changes in the mucosal surface, and has been shown to be effective in detecting neoplastic and dysplastic lesions in the upper aerodigestive tract, including the oral cavity, oropharynx, hypopharynx, larynx and esophagus.[4–10] Different patterns of intraepithelial microvasculature, such as dilated, tortuous, elongated, and twisted intraepithelial papillary capillary loop (IPCL) or IPCL destruction have been regarded as criteria for classifying a lesion as malignant in oral cavity.[4,8,10]

Inoue and coworkers revealed a correlation between IPCL alterations visualized by magnifying endoscopy and the invasion depth of superficial esophageal squamous cell carcinoma. They suggested that the NBI system offers advantages over ordinary white-light systems in accurately staging tumor depth.[11] Similar results were also reported by Kumagai et al.[12] To the best of our knowledge, no study on the use of endoscope with NBI system in predicting the depth of oral squamous cell carcinoma (OSCC) has been reported, nor has the correlation between pathologic characteristics (including tumor infiltration depth, grade of differentiation, pathologic TNM stage, lymphovascular invasion, and perineural invasion) and intraepithelial microvascular patterns been analyzed for OSCC. The aim of this study was to investigate the distinguishing features of intraepithelial microvascular morphology observed using NBI and analyze the relationship between these microvascular patterns and the pathologic characteristics of OSCC.

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Materials and methods

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital. A total of 80 patients with OSCC who underwent preoperative endoscopic examination with broadband white light (BWL) and NBI systems between April 2009 and December 2011 were enrolled in this study. Among them were 73 males (91.25%) and 7 females (8.75%), whose age ranged from 33 to 85 years; the average age was 54.18 ± 12.23 years. Records of patients with OSCC who underwent flexible endoscopy with BWL and NBI at the Department of Otolaryngology – Head and Neck Surgery of Chang Gung Memorial Hospital, Keelung were retrospectively reviewed. Before biopsy, examinations were carried out with ENF TYPE V2, TYPE VQ, and TYPE VT (Olympus Medical Systems Corp., Tokyo, Japan) NBI endoscope. One light source was for the standard optical filter (BWL) and the other was for the NBI system. A BWL light source emits visible white light with a broad optical bandwidth. An NBI light source emits light of wavelengths in the visible spectrum filtered from the illumination source, with the exception of narrow bands in the blue and green spectrum, which focus at 415 nm and 540 nm and coincide with the peak absorption spectrum of oxyhemoglobin and therefore insure that blood vessels are more pronounced when viewed in NBI mode. The examinations were initially performed with BWL illumination in wide view to observe the whole lesion and its surrounding mucosa. The same procedure was performed with NBI illumination, and the capillaries were analyzed in detail and results recorded. The examinations in the oral cavity were initiated at the right upper and lower lip labial mucosa and continued through the buccal mucosa, upper and lower gum mucosa, mucosa of the floor of the mouth, ventrolateral and dorsal tongue, retromolar mucosa, anterior tonsillar pillar mucosa, and palate mucosa; subsequently, the sequence was repeated on the left side. Tumor mass, ulcerative mucosa lesion, or mucosa lesion with irregular mucosa surface, abnormal hue or luster found under BWL was selected for further evaluation under NBI illumination.

The images were recorded and transferred to a hard drive on the computer. Clinical characteristics revealed under BWL were analyzed first, and the IPCL features observed under NBI illumination were analyzed subsequently. Informed consent was obtained from every patient. After endoscopic examinations, biopsy (scalpel with standard surgical blade No. 15) was performed under local anesthesia in the operating room. Patients who met the following criteria were enrolled: (1) patients with the primary tumor located in the oral cavity; (2) patients with histopathologically proven squamous cell carcinoma; (3) patients who received endoscopic examination with BWL and NBI before any surgical intervention (biopsy or surgery); (4) patients who completed tumor studies before radical surgery, including computed tomography (CT) or magnetic resonance image (MRI) of the head and neck, abdominal ultrasonography, bone scan, and chest roentgenogram; fine needle aspiration cytology or positron emission tomography (PET–CT) scan was optional depending on the clinical status of disease; (5) patients who underwent resection of the primary tumor, without prior head–neck or systemic oncological treatment at Chang Gung Memorial Hospital, Keelung; (6) patients who did not receive neck dissection as part of the initial treatment, postoperative follow-up should be longer than 2 years and no clinical regional metastasis was found. If more than one IPCL type was identified with NBI, the most advanced type detected was determined as the IPCL type of the lesion.

Each patient's chart records were reviewed, including their demographic data, morphology of the vascular architecture or the IPCL,[4,12,13] tumor localization, and pathological tumor characteristics (e.g., grade of differentiation, pathologic TNM classification, depth of tumor infiltration, perineural and lymphovascular invasion). The relationship between the pathologic characteristics and different IPCL types of microvasculature shown by NBI was analyzed for statistical significance.

Patients' histories related to use of betel quid, alcohol and tobacco were obtained during detailed questioning of the patients on their first visit to the otolaryngology clinic of the hospital. The criteria for a positive assignment were defined as previously described.[14] A past drinker was defined as an individual who exercised total abstinence from alcohol for 6 months or longer. Similar definitions were applied for past smokers or betel quid chewers.

Determination of neck nodal status

For patients who received neck dissection as part of the treatment modalities, the neck lymph node status (N-classification) was defined according to the pathology results. For patients who did not receive neck dissection, the N-classification was determined according to clinical palpation findings combined with image study (CT or MRI). PET–CT scan or fine needle aspiration cytology, if performed, could provide information for the N-classification. For patients who did not receive neck dissection (clinically preoperative evaluation without evidence of neck nodal metastasis), follow-up time longer than 2 years and no regional recurrence were confirmed before enrolment in this study.

Measurement of infiltration depth

Infiltration depth was measured by two experienced pathologists (Dr. L.-C. Chang and Dr. T.-Y. Hsieh) using a light microscope equipped with digital camera and computer software (original magnification, ×400; Nikon microscope with Olympus digital camera DP20, software: DP2-BSW, OLYMPUS Corporation 2006–2008, Japan). In cases of ulcerated or exophytic tumors in which the epithelium was beneath or above the level of adjacent normal epithelium, a line through the tumor at the level of basement membrane of the adjacent normal epithelium was constructed. This reconstructed mucosal surface was used to calculate the infiltration depth.[15] Infiltration depth was defined as the depth of the surface of the normal mucosa to the deepest portion of the tumor (millimeters).

Statistical analysis

Results are presented descriptively, with pathologic parameters related to different intraepithelial microvascular patterns grouped and analyzed using Fisher's exact test between groups for univariate analysis. The difference in tumor infiltration depth between different NBI microvascular patterns was calculated by analysis of variance (ANOVA). Receiver operator curve (ROC) analysis was performed to determine the infiltration depth cut-off point for the optimal prediction (highest score of sensitivity plus specificity) of different NBI microvascular patterns. The p-value is the probability of getting a correlation as large as the observed value by random chance, when the true correlation is zero. If the p-value is small, less than 0.05, the correlation value is significant. Statistical analysis was performed using MATLAB program version 7.12 (MathWorks Inc., Natick, MA).

Results

In cases of OSCC examined in our study, the microvasculature was clearly delineated with NBI illumination. The microvascular