

Prognostic impact of preoperative serum interleukin-6 levels in patients with early-stage oral squamous cell carcinoma, defined by sentinel node biopsy

HIROYUKI GODA¹, MASATO OKAMOTO², KOH-ICHI NAKASHIRO¹,
SATOSHI HINO¹, RYUICHI MURASE¹ and HIROYUKI HAMAKAWA¹

¹Department of Oral and Maxillofacial Surgery, Ehime University Graduate School of Medicine, Tōon, Ehime 791-0295;

²Department of Advanced Immunotherapeutics, Kitasato University School of Pharmacy, Tokyo 108-8641, Japan

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Abstract. Failure to detect recurrence and lymph node metastasis early represents a fundamental barrier to the improvement of survival rate in early stage oral squamous cell carcinoma (OSCC). The present study evaluated the association between serum interleukin-6 (IL-6) level and clinical outcomes in patients with early stage OSCC patients defined by sentinel node biopsy (SNB). A total of 53 patients with clinical stage I/II OSCC who underwent SNB were enrolled. SNB was determined by a radioisotope method, and was evaluated by histopathological examination and genetic analysis. Preoperative sera were measured for IL-6 by ELISA. In the clinical stage I/II patients, disease-free survival (DFS) was demonstrated to be higher in patients with negative SNB compared with patients with positive SNB. In total, 13 patients were demonstrated to exhibit lymph node metastasis by SNB or were reclassified to pathological stage T4 subsequent to analysis of the surgically resected specimens. Thus, 40 patients were diagnosed with early stage OSCC. Of these 40 patients, DFS of the patients with low serum IL-6 was significantly higher compared with the patients with high serum IL-6 ($P=0.012$). In 19 patients with negative SNB and low serum IL-6, the disease-free rate was 100%. These findings suggested that SNB staging and serum IL-6 level have a high prognostic value in patients with early stage OSCC. Additional investigation and longer follow-up times are warranted to improve understanding of the group of patients that may benefit from this procedure.

Introduction

Oral squamous cell carcinoma (OSCC), the most common type of head and neck carcinoma, represents the fifth most frequently occurring cancer worldwide (1). An estimated 263,900 new cases and 128,000 mortalities occurred globally in 2008 (2). Despite advances in surgery, radiotherapy and chemotherapy, little improvement in the relative survival has been observed in OSCC during the past 30 years (3).

Early-stage OSCC (clinical stage I or II) (4) is primarily managed with surgery. The nodal status of the cervical lymph nodes remains an important prognostic factor in OSCC (5-6). The presence of cervical lymph node metastasis reduces the survival of patients with SCC of the upper aerodigestive tract by up to 50% (7). Therefore, early detection of cervical lymph node metastasis is hypothesized to improve survival. However, the diagnostic accuracy of lymph node metastasis using imaging tools including ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) is ~70% (8). Furthermore, patients with delayed neck node metastases generally exhibit a poor prognosis (9).

Sentinel node biopsy (SNB) has been demonstrated to be an oncologically safe staging modality in patients with early stage OSCC, allowing for an individualized and minimally invasive treatment of the neck, and significantly affecting tumor control and survival (10).

However, patients with tumor recurrence and metastases generally exhibit poor prognosis, and predictive biomarkers that identify the risk of tumor relapse may become a powerful tool for follow-up and development of effective treatment plans for these patients (11-13). Sera derived from patients with early stage OSCC were previously examined for multiple cytokines using a multiplexed measurement system (14) and serum interleukin-6 (IL-6) level was revealed to negatively correlate with a favourable outcome in these patients. IL-6 is a multifunctional cytokine that functions in the regulation of inflammatory and immune responses. IL-6 is produced by a variety of cells, primarily monocytes, macrophages and several types of tumor cell during infection and immunological challenge (15). Previous studies have revealed that IL-6 is involved in cancer progression,

Correspondence to: Dr Hiroyuki Goda, Department of Oral and Maxillofacial Surgery, Ehime University Graduate School of Medicine, 454 Shitsukawa, Tōon, Ehime 791-0295, Japan
E-mail: hiro9832@m.ehime-u.ac.jp

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including proliferation, angiogenesis and lymphangiogenesis in several types of cancer, including OSCC (16-19), and worsens cancer prognosis (20-22). The aim of the present study was to determine the function of serum IL-6 concentration in patients with early-stage OSCC defined by SNB.

Materials and methods

Patients. The present study was approved by the medical ethics committee of Ehime University Hospital (Tōon, Japan) for the Protection of Human Subjects. Informed written consent was obtained from all patients, and the collection of samples was approved by the Institutional Review Board.

A total of 53 patients with clinically diagnosed T1-2N0 OSCC (4) scheduled for radical resection of their tumor(s) and SNB between September 2006 and June 2013 were eligible to participate in the present study. The primary sites were the tongue, gingiva, oral floor, buccal mucosa and lip in 50, 36, 8, 5 and 1% of tumors, respectively. The histological differentiation of the samples was classified in accordance with the World Health Organization classification (23). The mode of cancer invasion was classified into five grades according to the classification proposed by Yamamoto and Kohama (YK classification): YK-1, well-defined border; YK-2, cords, less marked border; YK-3, groups of cells, no distinct border; YK-4C, diffuse invasion, cord-like type; and YK4-D, diffuse invasion, widespread type (24).

Serum collection and IL-6 ELISA. Prior to surgery, serum was collected and immediately frozen at -80°C until being used for the IL-6 assay. Serum IL-6 levels were analyzed using a human IL-6 ELISA kit (cat. no. S6056; BioLegend, Inc., San Diego, CA, USA) according to the manufacturer's protocol. The cut-off value of 20.0 pg/ml was selected based on the receiver operating characteristic (ROC) curve.

SNB. SNB has received considerable attention for its role in the decision of whether to perform neck dissection (11). The day prior to surgery, a total of 0.4 ml (74 MBq) ^{99m}Tc-tin colloid was slowly injected around the primary tumor at four points. The sentinel lymph node (SN) was detected by scintigraphy 2 h following this. The middle and lower portions of the face were covered with a lead plate to reduce shine-through, and four Eppendorf mini tubes, each containing 30 μl radioisotope solution, were placed at the chin, the mandibular angle and the anterior and posterior ends of the clavicle to serve as point markers. A small incision was made in the skin above the SN at operation. Radioactivity of the SN was measured with the gamma probe Neo 2000 (Navidea Biopharmaceuticals, Dublin, OH, USA). The SN was rinsed quickly in saline solution to remove attached blood, the peripheral adipose tissue and connective tissue were carefully stripped off, and the SN was subsequently divided in two. One half of each lymph node was examined through sectioning (200 μm thick) of the maximal cut surface of the specimen, stained with hematoxylin and eosin and diagnosed as metastatic or non-metastatic. The remaining half of the lymph node was analyzed by one step nucleic acid amplification (OSNA) to calculate the cytokeratin-19 (CK-19) mRNA copy number as previously described (25). A previous study

Table I. Patient and tumor characteristics.

Clinicopathological characteristic	Number (%)
Number of patients	53
Age, median (range)	68.5 (40-91)
Sex	
Male	31 (58.5)
Female	22 (41.5)
WHO (grade)	
1	38 (71.7)
2	12 (22.6)
3	3 (5.7)
Stage	
1	24 (45.3)
2	27 (50.9)
3	0
4	2 (3.8)
SNB	
+	10 (18.9)
-	43 (81.1)
Pathological T4 (bone invasion)	7 (13.2)
Mode of cancer invasion	
YK-1	2 (3.8)
YK-2	7 (13.2)
YK-3	27 (50.9)
YK-4C	16 (30.2)
YK-4D	1 (1.9)
Primary sites	
Tongue	24 (45.3)
Gingiva	23 (43.4)
Floor of the mouth	4 (7.5)
Buccal mucosa	1 (1.9)
Lip	1 (1.9)

WHO, World Health Organization; SNB, sentinel node biopsy; YK, Yamamoto and Kohama classification.

classified OSNA levels in OSCC as follows: CK19 mRNA copies <300/μl was designated as negative, and >300/μl as positive (25). Neck dissection was performed in metastatic cases, determined by either histopathological or genetic examination.

Statistical analysis. Kaplan-Meier curves and log-rank tests were utilized to assess any differences in survival times between the treatment groups. A multivariate analysis was performed to evaluate impact factors on survival using Cox's proportional hazards regression model. Differences between the groups of categorical data were analyzed using two-sided Fisher's exact tests. P<0.05 was considered to indicate a statistically significant difference. GraphPad Prism statistical software version 5 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis.

Table II. Summary of SNB results.

Cases	n	Identification rate (%)	SN positive rate (%)	False negative rate (%)	Accuracy (%)
All	53	100 (53/53)	18.9 (10/53)	28.6 (4/14)	92.5 (49/53)
pT1-2	46	100 (46/46)	13 (6/46)	40 (4/10)	91.3 (42/46)
pT3-4	7	100 (7/7)	57.1 (4/7)	0 (0/4)	100 (7/7)

SNB, sentinel node biopsy; p, pathological stage.

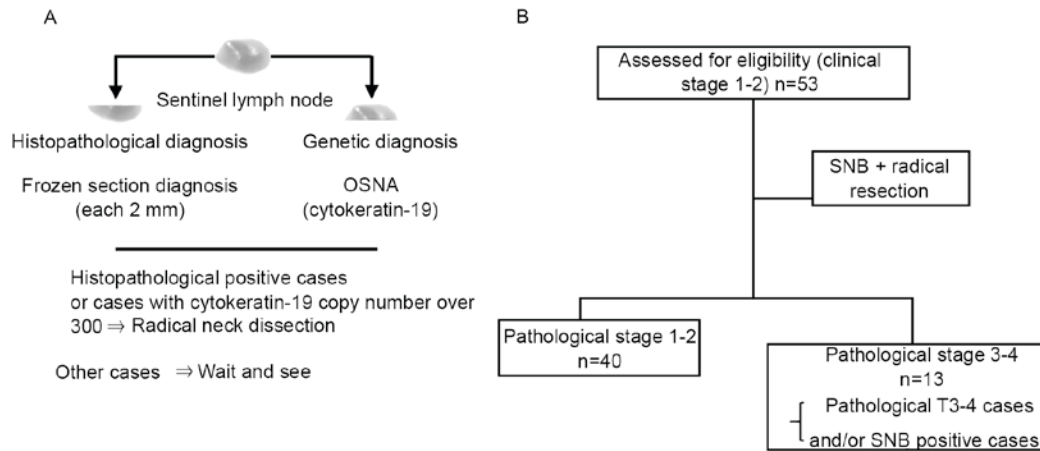


Figure 1. (A) SNB procedure and (B) diagnosis of pathological stage. SNB, sentinel node biopsy; OSNA, one step nucleic acid amplification.

Results

SNB. Table I lists the patient and tumor characteristics. A total of 53 patients with clinically diagnosed T1-2N0 OSCC undergoing SNB were included in the present study. There were 31 male and 22 female patients, with a mean age of 69 years (range, 40-91 years). The mean number of SNs identified was 1.9 (range, 1-5). In total, 19 patients had 1 SN removed, 22 patients had 2 SNs, 11 patients had 3 SNs and 1 patient had 5 SNs removed. The SNB results are summarized in Fig. 1 and Table II. The SN metastases were pathologically and genetically diagnosed by the SNB specimens in 18.9% of cases (10/53 cases). The identification rate by SNB was 100% (53/53 cases), accuracy was 92.5% (49/53 cases), the SN positive rate was 18.9% (10/53) and the false negative rate was 28.6% (4/14 cases). In 46 patients with pathological T1-2, the identification rate by SNB was 100% (46/46 cases), accuracy was 90.3% (42/46 cases), SN positive rate was 13% (6/46) and the false negative rate was 29% (1-sensitivity). In patients with pathological T3-4, the identification rate of SNB was 100% (7/7 cases), the accuracy was 100% (7/7 cases), the SN positive rate was 57.1% (4/7) and the false negative rate was 0%. Of these 53 patients, 13 (24.5%) were excluded from the pathological stage 1-2 group due to presence of pathological T 3-4 stage (including bone invasion) tumor and SN metastasis (Tables II and III).

Association between the SNB status of patients with OSCC and serum IL-6 levels. The cut-off value of 20.0 pg/ml was selected based on the ROC curve. ELISA was performed for IL-6 in the 53 patients exhibiting cT1-T2 and evaluated for association

with lymph node metastasis. Analysis revealed no association between SN metastasis and IL-6 level (P=0.73; Table IV).

Kaplan-Meier survival plots comparing SNB and serum IL-6 levels. Kaplan-Meier survival plots comparing the overall survival (OS) and disease-free survival (DFS) fractions of each group are depicted in Fig. 2. OS (Log rank P=0.03) and DFS (Log rank P=0.02) were significantly lower among the patients whose SNB status and tumor bone invasion status were positive (Fig. 2A and B). Among the 53 patients, the 24 patients of the low serum IL-6 group tended to survive longer (P=0.33 in OS, P=0.047 in DFS), but the difference was not significant for OS (Fig. 2C and D). A total of 13 patients were diagnosed with lymph node metastasis by SNB or were reclassified to pathological stage T4 from evaluation of the surgically resected specimen. Thus, 40 patients were strictly defined as pathological stage 1-2 OSCC. Among them, the OS of patients with low serum IL-6 levels was not statistically significant different compared with those with high serum levels (P=0.24; Fig. 2E) but DFS of the patients with low serum IL-6 levels was significantly longer compared with those with high serum IL-6 (P=0.012; Fig. 2F). For patients with pathological stage 1-2 tumors strictly defined by SNB and pathological examination, OS and DFS were 100% in the low serum IL-6 group in the follow-up period (Fig. 2E and F).

Discussion

The present study demonstrated that staging by SNB and preoperative serum IL-6 level exhibited a high prognostic value

Table III. Metastasis in SNB positive and negative cases.

Cases	Metastasis (+)	Metastasis (-)	Total
All	14	39	53
SNB (+)	10	0	10
SNB (-)	4	39	43
pT1-2	10	36	46
SNB (+)	6	0	6
SNB (-)	4	36	40
pT3-4	4	3	7
SNB (+)	4	0	4
SNB (-)	0	3	3

SNB, sentinel node biopsy; p, pathological stage.

Table IV. Serum IL-6 levels in SNB negative and positive cases.

Serum IL-6	SNB negative	SNB positive
High	25	5
Low	18	5

SNB, sentinel node biopsy; IL-6, interleukin-6.

in patients with OSCC. In patients with early-stage OSCC, the nodal status of the cervical lymph nodes remained an important prognostic factor. Advances in imaging technology have made additional techniques available, including US, CT, MRI and PET imaging. However, the diagnostic ability of US, CT and MRI is primarily based on node morphology and size criteria, with nodes <10 mm generally considered to not harbour metastasis. Attempts with functional imaging using PET scanning were also revealed not to be efficient in detecting occult cervical metastasis. Since the early 1980s, treatment of patients with clinical N0 OSCC has evolved from observation to elective neck dissection for all but the earliest-stage types of cancer of the oral cavity, with elective neck dissection becoming the standard of care at the majority of institutions treating a large number of patients with cancer in the 1990s (7). However, elective neck dissection is a surgical procedure that was revealed to be over-treatment for ~70% of patients with cN0, who were revealed to have a pathological node-negative neck (8). Observation was the primary therapeutic modality employed in the management of patients with N0 neck tumors, yet it soon became evident that ~30% of observed patients developed cervical node metastasis despite tumor control at the primary site (26-28).

An SN is the first lymph node(s) to which cancer cells are most likely to spread from a primary tumor. SNB may be used to help determine the extent of the disease, and to stage and guide the use of adjuvant radiation and chemotherapy to reduce disease recurrence. As SNB involves less extensive surgery and the removal of fewer lymph nodes compared with neck dissection, the risk of adverse effects is lower. In

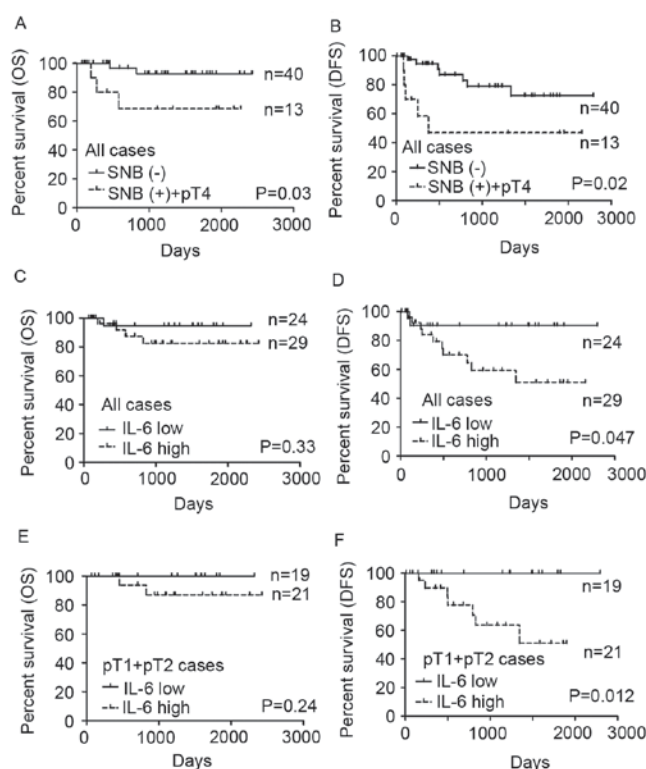


Figure 2. Kaplan-Meier survival plots comparing (A) SNB and OS, (B) SNB and DFS, (C) serum IL-6 levels and OS, (D) serum IL-6 levels and DFS in all cases, and (E) serum IL-6 levels and OS and (F) serum IL-6 levels and DFS in patients with pT1-2 oral squamous cell carcinoma. SNB, sentinel node biopsy; OS, overall survival; DFS, disease-free survival; IL-6, interleukin-6; p, pathological stage.

the present study, the SNB positive rate was 18.9% (10/53), but the delayed neck metastasis was 7.5% (4/53). Consistent with previous reports of cervical metastasis, the present study revealed a lymph node metastasis rate of 26.4%.

The present results suggested that SNB may improve the prognosis of patients with OSCC, with a significantly higher OS (P=0.03) and DFS (P=0.02) in patients whose SNB status was negative. However, a few cases of delayed neck metastasis and local recurrence were observed, even in SNB negative cases. A possible cause may be the lack of rapid and accurate intraoperative detection of metastatic disease in the SN(s) (29,30). In addition, the occurrence of a second primary tumor is 3-7% higher per year in patients with OSCC compared with other malignancies (31). Therefore, the identification of suitable and reliable biomarkers is essential for achieving early detection and treatment, which may reduce mortality rates in patients with OSCC. However, several serum cytokines were previously examined in patients with OSCC by using a multiplexed measurement system, revealing that the serum IL-6 level tended to negatively correlate with favorable outcome in these patients, with OS and DFS rates of 100% in patients with early-stage OSCC diagnosed by SNB with low levels of serum IL-6. Furthermore, adjuvant and/or neo-adjuvant therapies may be administered to patients with high levels of serum IL-6, despite having only early-stage OSCC. In this previous study, there were 4 false negative cases (delayed neck metastasis). It was impossible to analyze the statistical significance with respect to correlation between the serum IL-6 level and frequency of the delayed neck metastasis in SNB

negative cases as there were few applicable cases. Therefore, this requires additional investigation.

Previous studies have revealed that stromal fibroblasts isolated from various types of cancer produced a significant amount of IL-6, which induced tumor proliferation, invasion, migration and angiogenesis (19,32,33). An on-going prospective study may further elucidate the prognostic and predictive significance of IL-6, which may warrant future clinical trials of an IL-6 inhibitor for patients with high-risk OSCC.

In conclusion, the present study revealed that SNB staging and preoperative serum IL-6 level have a high prognostic value in patients with OSCC, and IL-6 may be associated with poor clinical outcome. Future studies are required to increase understanding of the biological significance underlying the association of IL-6 with OSCC and the tumor microenvironment, including how these findings may be combined with other treatment approaches, including radiation and chemotherapy, which may contribute to the continued improvement of OSCC treatments.

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