



Contents lists available at [ScienceDirect](#)

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp



Original Research

Relationship between proliferative activity of tumor cells and the enlargement pattern of metastatic lymph nodes in oral squamous cell carcinomas

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ARTICLE INFO

Article history:

Received 9 February 2016
Received in revised form 2 September 2016
Accepted 26 September 2016
Available online xxx

Keywords:

Oral squamous cell carcinoma
Lymph node metastasis
Ultrasonography
Ki-67
N0 neck cancer

ABSTRACT

Objectives: This study was conducted to investigate the time-course changes in the size of metastatic lymph nodes and determine their relationship with the histological features of oral squamous cell carcinomas (OSCC).

Methods: Thirteen metastatic lymph nodes from 13 OSCC patients were examined in this study. The cervical lymph nodes were periodically followed up with ultrasonographic examination and diagnosed as metastatic when the minimum axis of the nodes (size) reached ≥ 8 mm in three-dimensional measurements. Enlargement patterns of the metastatic nodes were compared with the histological features and proliferative activity of tumor cells by Ki-67 immunostaining of the primary tumors and nodes.

Results: The enlargement patterns of the metastatic lymph nodes were classified into two types: rapid enlargement (RE), which involved rapid increase in node size, and slow enlargement (SE), which involved slow increase and fluctuation in size. No significant differences in histological findings between the RE and SE types were observed in the primary tumors or metastatic nodes. On the other hand, Ki-67 scores in primary tumors and metastatic foci were associated with the enlargement pattern of the metastatic nodes, wherein metastatic nodes of the RE type showed significantly higher Ki-67 scores than those of the SE type.

Conclusions: The proliferative activity of primary tumors can be a valuable marker for predicting the speed of enlargement of metastatic lymph nodes. In addition, it may aid in reaching a decision regarding follow-up duration in N0 neck cancer patients.

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1. Introduction

Cervical lymph node metastasis is one of the major factors influencing the prognosis of patients with oral squamous cell carcinoma (OSCC) [1]. The management of N0 neck cancer patients involves selective neck dissection and/or watchful observation; early detection of metastatic nodes is essential for patients under watchful observation [2]. Despite several studies on the imaging modalities and criteria of nodal metastasis, a standardized criteria has not been set so far; thus, clinicians are required to conduct follow-up and

reach a diagnosis based on their own criteria [3–6]. Yuasa et al. suggested that the follow-up of neck cancer patients with ultrasonographic (US) examination be performed every month because of abrupt metastatic changes within the nodes [4]. However, there are no known biomarkers that affect the speed of enlargement of metastatic lymph nodes till date [7]. Therefore, the present study focused on examining the enlargement of metastatic lymph nodes using the US examination method. In addition, the relationship of lymph node enlargement with proliferative activity of tumor cells was assessed to assist with the early detection of metastasis and the subsequent establishment of follow-up schedules.

2. Methods

This study included 13 metastatic lymph nodes from 13 patients (7 males and 6 females; average age, 69.2 years; age range 57–85 years) with oral squamous cell carcinoma (OSCC). The primary tumor sites were tongue (nine patients), gingiva (two patients),

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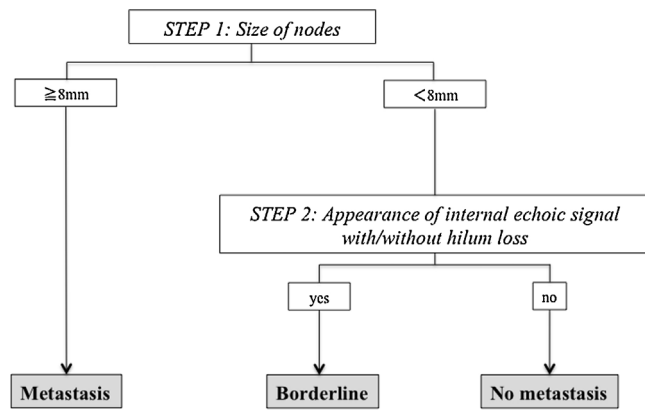


Fig. 1. Algorithm of the ultrasonographic diagnosis of metastasis in the regional lymph nodes of oral squamous cell carcinomas.

buccal mucosa (one patient), and the floor of the mouth (one patient). According to the TNM classification (UICC 2009), five patients presented with T1, seven with T2, one with T3, and one with T4 stage tumors.

No metastasis of the cervical lymph nodes was observed during initial examination in all patients who subsequently underwent resection of the primary tumors alone. Following surgery, they received punctual follow-up based on the wait-and-see policy used in the US examination method. B-mode US examination was periodically carried out for 2 years (monthly in the first year and bimonthly in the second year) after primary tumor resection. Lymph nodes detected during initial US examination were continuously monitored for the size and presence of internal echoic signs. Diagnosis of nodal metastasis was reached according to the algorithm shown in Fig. 1. Briefly, when the size of the nodes reached ≥ 8 mm, the nodes were diagnosed as metastasis. The size of the lymph node was defined as the minimum axis in three-dimensional measurements using the US method according to the Furukawa's report [6]. Lymph nodes with irregular internal echoic signals and/or loss of the hilum were judged as borderline.

Histological differentiation (WHO 2005) and invasion type of the primary tumors [8], area of tumor cells, degree of keratinization, existence of cystic changes, and occurrence of fibrosis in the metastatic nodes were examined histologically. Furthermore, the proliferative activity of tumor cells was assessed by Ki-67 immunostaining; biopsy specimens of primary tumors and surgical specimens of neck dissection of latent nodal metastasis were used for the Ki-67 staining. Briefly, 3- μ m-thick paraffin sections were deparaffinized in xylene, and immersed sequentially in graded concentrations of ethanol. Antigen retrieval was performed using citrate buffer (0.01 M; pH 6.0) in an autoclave at 121 °C for 15 min. After blocking the nonspecific binding sites using normal goat serum (10%), the sections were incubated with the Ki-67 antibody (MIB-1 diluted 1:400, Dako, Denmark) overnight at 4 °C in a humidified chamber. Immune complexes were detected using 3,3'-diaminobenzidine tetrahydrochloride.

Quantitative analysis of Ki-67 staining was carried out at the invasion front in primary tumors and metastatic nodes. Nuclear staining of any intensity was judged as positive [9]. Ki-67 positive cells were counted at a magnification of $\times 400$ in five fields selected at random. More than 1000 tumor cells were assessed for positive or negative immunostaining, and the Ki-67 score was obtained by calculating the percentage of positively stained tumor cells among the total number of tumor cells. Finally, the histological findings and proliferative activity of the primary tumors as well as the metastatic foci of nodes were compared with the time-course changes in the size of the metastatic node.

For the statistical analysis, χ^2 -test was used to examine the relationships between enlargement type and clinico-pathological parameters, except for patient age and Ki-67 scores. Differences in age were evaluated using student's *t*-test, whereas Spearman rank correlation test was used to compare the Ki-67 scores between primary tumors and metastatic foci.

3. Results

The time-course changes in the size of the 13 metastatic lymph nodes are shown in Fig. 2. The x axis indicates the duration after resection of primary tumors, and the y axis indicates the size of the nodes. The initial baseline size (BL) was measured during the first US examination, which was performed 4 weeks before surgical resection of the primary tumors in all patients. The duration between initial examination and diagnosis of metastasis ranged from 10 to 104 weeks. Two different patterns in metastatic lymph node enlargement were noted; the first pattern involved a rapid increase in size within a short period just before the lymph nodes met the metastasis criteria (LN#1–7) and was named as the rapid enlargement type (RE type). The second pattern was a slow increase in size (slow enlargement type; SE type), where the nodes gradually enlarged and often showed a temporary decrease in size before meeting the criteria for metastasis (LN#8–13).

The clinical characteristics and histological features of the primary tumors are shown in Table 1. The primary tumors were found to be well differentiated in eight, moderately differentiated in three, and poorly differentiated in two cases. The invasion type at the advancing front of the primary tumors was expansive in three tumors, intermediate in seven, and infiltrative in three.

Histological findings of the 13 metastatic nodes are summarized in Table 2. The area occupied by tumors cells within the nodal parenchyma was classified as $\sim 1/3$, $1/3$ – $2/3$, and $2/3$ – $3/3$. The tumors cells were observed within $\sim 1/3$ of the parenchyma in only one node, whereas in the remaining 12 nodes the tumor cells occupied more than $1/3$ of the area of the parenchyma. The degree of keratinization in the tumor nests of the metastatic foci was marked in five, moderate in two, and slight in six nodes. Cystic changes in the metastatic tumors were observed in 11 nodes. Cystic cavities were filled with exfoliated keratin layer and degenerated tumor cells, and sometimes occupied wide areas within the metastatic foci. Stromal fibrosis in the metastatic foci was marked in six nodes, slight in three, and undetected in four nodes.

Ki-67 scores in the primary tumors and metastatic foci are shown in Table 3. The average scores \pm standard deviation were 36.4 ± 10.1 and 32.0 ± 18.4 in the primary tumors and metastatic foci, respectively. There was a positive correlation between Ki-67 scores of the primary tumors and those of the metastatic lymph nodes (Fig. 3; $r = 0.753$, $y = 1.3466x - 17.064$). Next, we analyzed the correlation between enlargement type and clinical and histological variables. As shown in Table 4, we did not find any clinical or histological factors that were significantly related with the different enlargement types of metastatic lymph nodes. Finally, Ki-67 scores of primary tumors and metastatic foci were compared with the enlargement type of the metastatic nodes. As shown in Fig. 4, metastatic nodes of the RE type showed significantly higher scores than those of the SE type in both primary tumors ($p = 0.0177$) and metastatic foci ($p = 0.0182$).

4. Discussion

The status of the cervical lymph nodes is an important prognostic factor in patients with OSCC. Kalnins et al. [10] reported that the 5-year survival rate was reduced by approximately 50% in OSCC patients with cervical node metastasis compared to those without

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