99mTc(V)-dimercaptosuccinic acid scintigraphy in detecting neck metastases in oral squamous cell carcinoma with clinically negative necks

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SUMMARY

This study compares the accuracy of 99mTc(V)-dimercaptosuccinic acid (99mTc(V)-DMSA) Single Photon Emission Computer Tomography (SPECT) by intravenous or local injection in the detection of occult cervical lymph node metastases in clinically N0 squamous cell carcinoma of the oral cavity. Fifty-eight previously untreated patients without clinically detectable cervical metastases were included in the study and were divided into two groups. Twenty-eight patients were in the intravenous injection group and 30 patients were in the local injection (around primary tumors) group. Both groups received 99mTc(V)-DMSA, and 99mTc(V)-DMSA SPECT was performed on all patients. All isolated lymph nodes in neck dissection specimens were stained using H & E. The sensitivity, specificity, and accuracy for intravenous injection of 99mTc(V)-DMSA was 62.5%, 95.0%, and 85.7%, respectively, and 84.6%, 82.4%, and 83.3%, respectively, for local injection. SPECT scan of 99mTc(V)-DMSA injected around the tumor is a simple and efficient approach to detecting metastatic lymph nodes in clinically N0 patients with oral cancer.

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Introduction

Neck dissection is generally performed when patients with oral carcinoma present with clinically positive necks. In the case of occult cervical lymph node metastases, however, treatment of the neck is controversial because of the difficulty in diagnosis by palpation and various imaging procedures, such as CT, MRI, or ultrasonography. Although pathological biopsy of sentinel lymph nodes can solve this clinical problem, it is complicated by the possibility of misdiagnosis and emission, as well as by radioactive contamination and prolonged operation times. Dimercaptosuccinic acid (DMSA), similar to PO₄⁻ in structure and property, is involved in the metabolism of phosphoric acid and phospholipids, high levels of which characterize strong cancer cell to proliferation. 99mTc(V)-DMSA was routinely intravenously injected into patients and accumulated at the primary foci and metastatic lymph nodes in the neck. Watkinson showed that metastatic lymph nodes in the neck were too small to be detected by 99mTc(V)-DMSA SPECT, and the sensitivity and accuracy was less than that of CT. According to the injection method of sentinel lymph node biopsy (SLNB), we injected 99mTc(V)-DMSA around the primary tumor and assumed that 99mTc(V)-DMSA might drain to the lymph nodes via lymphatic vessels, remain in the suspected lymph nodes, and be imaged by SPECT; this method is different from the currently used sentinel biopsy mechanism. The purpose of this study was to compare two methods of applying DMSA, namely intravenous and local injection, with regards to feasibility and accuracy in detecting neck lymph metastases.

Patients and methods

Patients

A total of 58 patients with oral squamous cell carcinoma were enrolled from June 2002 to January 2006. All patients were diagnosed as having negative necks by physical and radiological examination. The intravenous injection group had 28 patients (males = 18, females = 10). Ages ranged from 28 to 77 years, with a mean age of 57.1 years. The local injection group had 30 patients (male = 19, females = 11). Ages ranged from 24 to 76 years, with a mean age of 56.4 years. Primary sites and TNM stages are shown in Table 1.

Labeling and imaging

555 MBq (15 mCi) of 99mTc(V)-DMSA were injected in the intravenous group, while the local injection group received 37 MBq (1 mCi) of 99mTc(V)-DMSA at four different sites into the perimeter of the tumor and the contra-lateral sites. SPECT scan was performed with the use of a Vertex dual-head gamma camera (AOAC Laboratories, Milpitas, CA) 4 h after injection of 99mTc(V)-DMSA in the intravenous group and 2 h after injection of 99mTc(V)-DMSA in the local group. Radiochemical purity was higher than 95%.
compared with the pathological examination. The neck was regarded as lymph node positive, and these results were recorded. Abnormal accumulation of nuclide in the neck region (for bilateral metastases). Radioactive intussusception ROI was calculated by drawing regions of interest (ROI) over the area of increased uptake corresponding to the suspect metastatic lymph node and the contra-lateral normal area, or the adjacent normal region (for bilateral metastases). Radioactive intussusception ROI values were recorded. Abnormal accumulation of nuclide in the neck was regarded as lymph node positive, and these results were compared with the pathological examination.

A 99mTc(V)-DMSA SPECT scan was performed on all patients before operation. Neck dissection at levels I–III and I–V was carried out in all patients. Five patients with a midline tumor underwent bilateral neck dissection, resulting in dissection of 63 neck sites (Table 2). All isolated lymph nodes in neck specimens were examined by hematoxylin–eosin staining. Suspected enlarged nodes (more than 1 cm in diameter) were analyzed in serial section with 50 μm intervals and HE staining, which microscopically exhibited lymph reactive proliferation.

**Table 1**

<table>
<thead>
<tr>
<th>Primary site</th>
<th>Intravenous group</th>
<th>Local group</th>
</tr>
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<tbody>
<tr>
<td>Tongue</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Gingival</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Oral floor</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Mandible</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Maxillary sinus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>30</td>
</tr>
</tbody>
</table>

**TNM stage**

<table>
<thead>
<tr>
<th>TNM stage</th>
<th>Intravenous group</th>
<th>Local group</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1N0M0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>T2N0M0</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>T3N0M0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>T4N0M0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>30</td>
</tr>
</tbody>
</table>

Acquisition was based on 360° noncircular rotation with 6° step angles, 60 s per frame, a 128 × 128 matrix, and a zoom factor of 1.33 (pixel size, 5 mm). Images were reconstructed with a Butterworth filter (order 5, cutoff 0.35, 1-pixel images) obtained in the sagittal, coronal, and transverse planes.

**Image analysis**

SPECT images of the head and neck were examined by two experienced nuclear medicine physicians and by the study authors. Coronal SPECT images of the two groups were analyzed quantitatively by drawing regions of interest (ROI) over the area of increased uptake corresponding to the suspect metastatic lymph node and the contra-lateral normal area, or the adjacent normal region (for bilateral metastases). Radioactive intussusception ROI values were recorded. Abnormal accumulation of nuclide in the neck was regarded as lymph node positive, and these results were compared with the pathological examination.

A 99mTc(V)-DMSA SPECT scan was performed on all patients before operation. Neck dissection at levels I–III and I–V was carried out in all patients. Five patients with a midline tumor underwent bilateral neck dissection, resulting in dissection of 63 neck sites (Table 2). All isolated lymph nodes in neck specimens were examined by hematoxylin–eosin staining. Suspected enlarged nodes (more than 1 cm in diameter) were analyzed in serial section with 50 μm intervals to identify possible metastases. 1627 nodes were revealed pathologically, of which 51 were malignant (Table 3).

**Statistical method**

The radioactive intussusception value of the ROI was analyzed statistically using paired two-tailed t-tests (SPSS for WINDOWS software, version 10.0).

**Table 2**

<table>
<thead>
<tr>
<th>Levels</th>
<th>Intravenous group</th>
<th>Local group</th>
</tr>
</thead>
<tbody>
<tr>
<td>I–III</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>I–V</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>32</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Lymph nodes</th>
<th>Metastatic lymph nodes</th>
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</thead>
<tbody>
<tr>
<td>Intravenous group</td>
<td>822</td>
<td>27</td>
</tr>
<tr>
<td>Local group</td>
<td>805</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>1627</td>
<td>51</td>
</tr>
</tbody>
</table>

**Results**

**Intravenous injection group**

Of the eight cases with pathologically diagnosed neck lymph node metastases in the intravenous injection group, five cases were positively screened by SPECT. Statistical analysis indicated that there was a significant difference in radioactivity between the normal and isotope-hot areas (paired t-test, t = 18.71, p = 0.00004). In 20 cases with pathologically negative lymph nodes, 19 cases were found to be negative by 99mTc(V)-DMSA SPECT. The sensitivity, specificity, and accuracy for intravenous administration of 99mTc(V)-DMSA were 62.5% (5/8), 95.0% (19/20), and 85.7% (24/28), respectively.

**Local administration group**

In the local administration group, 11 of 13 cases with pathologically diagnosed lymph node metastases were positively detected by SPECT, whereas 14 of 17 cases without pathologically diagnosed lymph node metastases cases were negatively diagnosed by SPECT. Statistical analysis showed a significant difference in radioactivity between the normal and isotope-hot areas (paired t-test, t = 8.182, p = 0.0000). The sensitivity, specificity, and accuracy for intravenous administration of 99mTc(V)-DMSA were 84.6% (11/13), 82.4% (14/17), and 83.3% (25/30), respectively.

Intravenous administration of 99mTc(V)-DMSA could detect the smallest 1 cm-in-diameter lymph nodes, while local administration reached the minimum 0.5 cm nodes. The suspected lymph nodes (more than 1 cm in diameter) of one false positive case in the intravenous administration group and three false positive cases in the local administration group were pathologically negative by serial section with 500-μm intervals and HE staining, which microscopically exhibited lymph reactive proliferation.

**Discussion**

A negative neck has several different meanings. The neck may negative to physical examination (palpation), to radiological evaluation, or to pathological interpretation. A clinically negative neck (cN0) means no clinical or radiological signs of neck metastases. Staging by palpation has been demonstrated to be inaccurate, while various imaging procedures have limits. Van den Brekel's comparative study showed that the accuracy of CT, MRI, and ultrasound-guided fine needle aspiration cytology was 66%, 75%, and 86%, respectively.1

It was reported that the risk of cervical nodal metastases in N0 patients with oral cancer was between 12% and 50%, with a medium value of 33%.10–13 Remarkably, it is inappropriate for more than 50% patients with lymph node metastases to receive neck dissection. Controversy continues regarding cN0 neck management. Some surgeons treat cN0 patients with a “wait-and-see” policy.14

Some studies stated that patients with T1-2 N0 tumors received definitive chemoradiation as an alternative to surgery, and achieved cure rates as high as 80%. Conversely, in the case of positive lymph nodes, surgery followed by radiotherapy or chemoradiation would be the treatment of choice.15 However, the majority of T1 and T2 tumors of the oral cavity are treated with initial surgical resection because of radiation complications such as xerostomia, dysphagia, caries, and osteoradionecrosis.16,17

Since Cabanas described the penile cancer sentinel lymph node (SLN) concept, it has been applied to and proven in the management of breast cancer and melanoma.18,19 Identifying and analyzing the SLN may aid in the selection of patients who will benefit...
from further surgical treatment of the neck. The SLN biopsy is a
good and novel approach to clarifying cervical lymph node metas-
tases. At the Second International Conference on Sentinel Node
Biopsy, 366 (97%) of 379 patients with cN0 disease were reportedly
identified for SLN, among which 103 (29%) cases were positive for
lymph node metastases. The sensitivity was between 80% and
100%, with a medium value of 90.3%.4

Neck SLN biopsy sheds light on determination of the operation
mode and in some sense improves clinical treatment for patients
with cN0 carcinoma; however, some problems still exist. First,
the head and neck region has approximately 300 lymph nodes,
which challenges SLN localization. Therefore, dissection of selected
lymph nodes will not necessarily reflect all head and neck lymph
nodes.2 Second, using isotope labeling to detect and dissect the
SLN is difficult because the primary tumor site of head and neck
carcinomas is close to the initial lymph nodes, which are easily
metastasized.3 Third, radioactive contamination should be empha-
sized, despite the short half life time of 99mTc and weak penetration
of the γ-rays released from 99mTc. Fourth, this approach can not di-
rectly detect lymph node metastases, and pathological diagnosis
would prolong operation times.4,20 For these reasons, neck SLN
biopsy can not be easily or widely used in clinics.

99mTc(V)-DMSA is widely used in detecting lung cancer, liver
cancer, breast cancer, and osteosarcoma because of its high affilia-
tion to tumor cells. In the present study, 99mTc(V)-DMSA was lo-
cally injected quadruply around oral carcinoma and SPECT was
performed 2 h later. Radioactive 99mTc(V)-DMSA accumulation
indicated risk of lymph node metastases. Local isotope administra-
tion might compensate for the weakness of SLN biopsy, as above.
Sensitivity was 84.6% higher than that of intravascular administra-
tion (62.5%), and consistent with the SLNB.2–6 Blue dye or a radio-
tracer, such as sulfur colloid, used in SLNB remained in lymph
nodes nonspecifically, while 99mTc(V)-DMSA remained in meta-
static lymph nodes only selectively. Local injection of 99mTc(V)DMSA can be used in clinical practice to determine lymph node
metastases.

The minimum lymph node diameter detected using intravenous
administration was 1 cm. The maximum lymph node diameters
were 1.2 cm, 1.1 cm, and 0.5 cm, respectively, for three false nega-
tive cases. In contrast, the minimum lymph node diameter de-
tected using local administration was 0.5 cm, and the longest
lymph nodes diameters were less than 0.5 cm for two false nega-
tive cases. Radioactive reagents enter lymph nodes via blood. It
was easy to determine large lymph nodes with metastases, but dif-
ficult to determine small, positive nodes. We speculated that radio-
active substances enter lymph nodes through lymph products at
amounts large enough to be detected by SPECT; this was the case
even for small nodes using local administration.

Our method largely met the challenge of confirming local
lymph node metastases of oral carcinoma, but mapping metastases
distribution was difficult due to the low resolution of nuclide
images. It has been documented that oral squamous carcinoma
generally metastasizes to levels I, II and III, but rarely to levels IV
and V, which is the theoretic basis for supraomohoid neck dissec-
tion.2,13 Therefore, it is more important and significant to identify
metastatic lymph nodes than to localize them.

Although the SPECT resolution is lower than those of CTs and
MRIs, it could be employed to discriminate between the upper,
middle, and lower regions of the neck. Figure 1 indicates left orophar-
yngeal carcinoma with bilateral lymph node metastases in the
submandibular region, and Figure 2 shows that a patient with
left tongue carcinoma was accompanied with lymph node metas-
tases in upper region of the neck.

Hamakawa41 thought that the minimum axial length of lym-
phatic metastases was 1.36 ± 0.85 mm and consecutive sections
within 1 mm intervals can omit less than 1 mm metastases. How-
ever, appropriate intervals for semiserial sectioning within 500 μm
intervals can equal complete serial sections. In both venous and lo-
cal administration groups, the enlarged lymph nodes (more than
1 cm in diameter) of four false positive cases were analyzed in se-
rial sectioning at 500 μm intervals; no metastatic cancer cells were
found, but lymph reactive proliferation was apparent. Until now, it
is believed that DMSA, similar to PO4
− in structure and property,
was involved in the metabolism of phosphoric acid and phospho-
lipids, high levels of which characterize strong cancer cell prolifer-
ation. Further investigation is required to determine whether
strong metabolism of fatty acids occur in lymph nodes with reac-
tive proliferation.

This study was limited by a relatively small number of patients;
therefore, these results should be confirmed by further intensive
investigation.

Conflict of Interest Statement

None declared.

References

1. van den Brekel MWM, Casteljins JA, Stel HV, et al. Modern imaging techniques
and ultrasound-guided aspiration cytology for the assessment of neck node
metastases: a prospective comparative study. Eur Arch Otorhinolaryngol
1993;250:11–7.

2. Werner JA, Dünne AA, Ramaswamy A, et al. The sentinel node concept in head
and neck cancer: solution for the controverses in the N0 neck? Head Neck

3. Civantos FJ, Moffat FL, Goodwin WJ. Lymphatic mapping and sentinel lymphadenectomy for 106 head and neck lesions: contrasts between oral
cavity and cutaneous malignancy. Laryngoscope 2006;116(Part 3 Suppl. 2):
1–15.

sentinel node biopsy in mucosal head and neck cancer. Annal Surg Oncol

cancer of the oral cavity and oral pharynx: a diagnostic meta-analysis. Head
Neck 2005;27(9):739–47.

6. Minamikawa T, Umeda M, Komori T. Reliability of sentinel lymph node biopsy


