치의학석사 학위논문

The occult metastasis in tongue cancer patients with clinically N0 neck

임상적으로 림프절 전이가 없는 설암 환자에서 잠재성 림프절 전이에 대한 연구

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치의과학과 구강악안면외과학 전공

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-Abstract-

The occult metastasis in tongue cancer patients with clinically N0 neck

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Purpose

The aim of this study is as follows: (1) to analyze the incidence and clinical aspect of occult metastasis in patients who showed clinically N0 with tongue cancer (2) to find the relationship between clinicopathologic findings and occult metastasis (3) to find bio

marker associated with occult metastasis by immunohistochemistry (4) to propose a useful diagnostic method for choice of treatment.

Materials and Methods

Patients who visited and underwent surgery in Seoul National University Dental Hospital from 2000 to 2012 were included in this study. The patients were pathologically diagnosed with oral tongue cancer and were shown no cervical lymph node metastasis in pre-operative work up. (Clinical examination, MRI, Ultrasonography, and PET).The patients were divided to 3 groups (Elective neck dissection group, Watchful waiting group, Total group). The patients in the END group received glossectomy with elective neck dissection. Patients in the WW group received only glossectomy with watchful waiting. In the END group, after elective neck dissection, occult metastasis was investigated. In the WW group, neck recurrence was investigated in watchful waiting period. Lymph node metastasis of the Total group was a combined group consisting of patients with occult metastasis in the END group and neck recurrence in the WW group. We investigated the incidence of lymph node metastasis, location of lymph node metastasis (Level), treatment method, depth of invasion, differentiation, T-stage, age, and sex.

For immunohistochemistry, paraffin-embedded blocks of 41 cases of oral tongue cancer specimens were examined with antibody for VEGF-c, c-Met, Cox-2, Podoplanin, and ROR1.

Chi-square test and Fisher's exact test were used to evaluate the association between lymph node metastasis and clinicopathologic factors. Overall survival rates were evaluated using Kaplan Meier method and values were compared by log-rank test. The correlation between immunohistochemical finding and lymph node metastasis was analyzed with the chi-square test, Fisher's exact test. Statistical tests were performed using SPSS software (version 21) (IBM Corp., Armonk, Chicago, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Result

Clinical finding

81 of 109 patents were male and 28 female. The mean age was 54.4±15.4 years, ranging from 23 to 91. Among 71 patients who received elective neck dissection with glossectomy, occult metastasis was observed in 13 patients and neck recurrence was observed in 8 patients among the 38 patients who received

glossectomy only. As a result, the incidence of occult metastasis in the END group was 18.3%. The incidence of neck recurrence in the WW group was 21.1% and the incidence of occult metastasis in the total group was 19.3%.

Histopathologic findings

Patients in T2–4 group showed more occult metastasis than patients in T1 group, and this was statistically significant in the END group (P=0.017).

The group with thickness of greater than or equal to 3 mm showed more incidence of lymph node metastasis than the group with thickness of less than 3 mm. Depth of invasion greater than or equal to 3 mm in the Total group was statistically correlated with occult metastasis (P=0.022).

Moderate/poor differentiation group showed more incidence of lymph node metastasis than well differentiation group. However, all values were not statistically significant.

In the END group, occult metastasis was found in 4 patients at level I, 2 patients at level II, 4 patients at level III, and 1 patient at level IV. Two patients displayed occult metastasis at multiple levels (level II, III). In the WW group, lymph node metastasis was found in 2 patients at level I, 2 patients at level II, and 1 patient at level III.

Three patients displayed lymph node metastasis at multiple levels (level II, III; 2patients, level I, III, IV; 1patient).

Survival analysis

The 3- and 5-year overall survival rates of the WW group were 88.4% and 84.3%, and the rates of the END group were 75.8% and 71.9%, respectively. Patients in the WW group showed better survival rate than patients in the END group, although this was not statistically significant (p=0.068). Patients in the pN0 group showed better survival rate than patients in the pN (+) group (p=0.001).

Immunohistochemistry

Positive VEGF-c expression had significant relationship with occult metastasis in the Total group (p=0.043). Positive c-Met expression had significant relationship with occult metastasis in the Total group. (p=0.009) Positive ROR1 expression had significant relationship with occult metastasis in the Total group (p=0.003), in the END group (p=0.013). Other markers had no significant relationship with occult metastasis.

Conclusion

Patients with thickness of greater than or equal to 3 mm and with more advanced T stage showed more incidence of lymph node metastasis. Patients in pN0 group showed better survival rate compared to patients pN(+) group. VEGF-c, c-Met, ROR1 had statistically significant correlation with occult metastasis. VEGF-c, c-Met, ROR1arethoughtto be expressed nearly stage of lymph node metastasis.

This study showed relatively high incidence of occult metastasis and even showed skip metastasis in tongue cancer patients with N0 neck. By considering clinical, histological, and immunohistochemical factors, surgeon can determine whether END or WW. First of all, close follow up is important to obtain similar results between the WW group and the END group.

Keywords: Occult metastasis, Neck recurrence, Elective neck dissection, Watchful waiting, Immunohistochemistry

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I. Introduction

Head and neck cancer constitutes approximately 5% of all malignant tumors and its incidence is increasing. Oral tongue cancer constitutes approximately 31.9% of oral cancer.[1] Tongue cancer is the most common cancer among the types of oral cancer. Poor oral hygiene, drinking, smoking and stomatitis syphilitica may be the cause of tongue cancer.[2] Unlike other head and neck cancers, the vascular system and the lymphatic system are well developed in tongue. Therefore, incidence of cervical lymph node metastasis (LNM) is high.[3]

The influence of LNM on the survival of head and neck cancer patients has been well established. The existence of LNM is the most important prognostic factor for survival of head and neck cancer patients.[4-8] The average 5-year survival rate is more than 50% in patients without LNM, but only 30% in patients with LNM.[9]

The diagnostic methods include manual palpation, CT (computed tomography), MRI (magnetic resonance imaging), neck ultrasonography, PET (positron emission tomography), and so on. The final diagnosis is confirmed by histopathologic examination of

lymph nodes after neck dissection. Unfortunately, this LNM is often occulted when the diagnosis is made[3] Approximately 25% of occult metastasis is too small to be detected by imaging techniques.[10] Teichgraeber *et al.* reported that the incidence of occult metastasis was 35%.[11] Shah *et al.* reported that the incidence of occult metastasis was 35% after neck dissection in oral tongue squamous cell carcinoma.[12] 20–50% of occult metastasis has been found in oral tongue cancer patients.[3,13]

The treatment modality consists of watchful waiting after glossectomy, glossectomy with elective neck dissection (END), and radiation therapy. However, the treatments for patients with clinically negative (N0) head and neck cancer remains controversial about when and how to manage the neck in patients of N0 neck. A survey performed in the United States found a lack of consensus regarding the treatment of the N0 neck.[14] A similar finding was described in the European survey in Marburg, Germany.[15] There is no consensus on the treatment for patients with Clinically N0 neck, yet. Either elective neck dissection or watchful waiting policy has been the preferred treatment for oral tongue cancer patients among surgeons worldwide.[13,16]

Because LNM is often occulted before surgery, the new highly sensitive detection method, such as immunohistochemistry should

be developed. Numerous studies of immunohistologic markers such as VEGF-c, c-Met, COX-2, podoplanin, ROR1have been carried out so far.

Vascular endothelial growth factor is essential in angiogenesis and vasculogenesis. Two lymphangiogenic factors, VEGF-C and -D have been found and these factors promote lymphangiogenesis in animal models.[17-19] Lymphangiogenesis is a critical step in LNM. The increment of VEGF-C expression is related to the LNM in human thyroid, lung, prostate, gastric, colorectal, breast cancer, and melanoma.[20-22] But the exact role of VEGF-C in LNM of oral squamous cell carcinoma is still unclear.

c-Met is known as MET and hepatocyte growth factor receptor (HGFR). This protein is encoded by the MET gene (MET proto-oncogene, receptor tyrosine kinase). c-Met regulates cellular processes, cell function, and tissue homeostasis in mammalian development.[23] Also, c-Met can activate lymphangiogenesis which may cause LNM.[24,25] The c-Met pathway is activated in various cancers (kidney, liver, stomach, breast, and brain) and it attributes to poor prognosis, tumor aggressiveness, and resistance.[26,27] Recently, c-Met inhibitor has been studied in clinical trials as a new therapeutic target.[28] Some reports on head and neck cancer have described expression of c-Met as clinical parameters.[29] These

reports have shown correlation between overexpression of c-Met and advanced stage and LNM. therefore, it was suggested that c-Met has contributed to worse characteristics in head and neck cancer.[30)]

Cyclooxygenase (COX) is an enzyme responsible for the oxidation of arachidonic acid. COX has been expressed in two different isoforms (COX-1, COX-2). COX-1 links with prostaglandin production and influences various physiological effects such as vascular homeostasis, maintenance of renal blood flow, platelet aggregation, and gastric protection. COX-2 is expressed by cells in the inflammatory site, such as monocytes, synoviocytes, and macrophages. COX-2 is a significant factor in carcinomas of various tissues and it induces carcinogenesis through the increment of tumor survival.[31-33)] Overexpression of COX-2 affects cancer development, such as angiogenesis, invasiveness, and apoptosis.[31,34,35)] Also, COX-2 affects LNM in gastric, breast, lung, and head and neck cancers.[36-40)]

Podoplanin is a transmembrane glycoprotein, is composed of a 38-kDa type-1 transmembrane sialomucin-like glycoprotein. Podoplanin was found in 1996, by Wetterwald *et al.*[41)] It was called podoplanin because of its low level expression in podocytes of the renal corpuscle. Podoplanin is specifically expressed in lymphatic

endothelial cells.[42,43] Moreover, podoplanin influences lymphatic vessel formation but has no effect on formation of blood vessel.[42] Podoplanin knockout mice have defects in lymphatic system, reduction of lymphatic transport, dilation of lymphatic vessels, and congenital lymphedema.[44] Podoplanin is expressed in normal tissues as well as neoplastic tissues, And expression of podoplanin might be related to cell migration and invasion.[45,46]

Receptor tyrosine kinase-like orphan receptors (RORs) are transmembrane proteins with the receptor tyrosine kinase family. RORs were initially found in a cell line of neuroblastoma. Therefore, RORs were named as neurotrophic tyrosine kinase receptor-related proteins (NTRKR) in past.[47,48] The structure of RORs consists of an extracellular immunoglobulin-like domain, a cysteine-rich domain (Frizzled domain), and a transmembrane domain (Kringle domain). ROR1 is a transmembrane protein regulating skeletal and neuronal development, cell polarity, and cell migration.[49,50] ROR1 is expressed during embryogenesis. And it is generally found in embryonic tissue and is generally lacking in adult tissue.[51,52] Many studies have shown that ROR1 was overexpressed in human cancers.[53-57] And it can serve as a target for cancer therapy.[58-60] Wnt5a (a ligand of ROR1) is involved in the ROR1-dependent signaling pathway, enhancing cancer cell growth.[54,55]

ROR1 participates in progression of many blood, solid cancers, inhibition of apoptosis, induction of EGFR signaling, and epithelial mesenchymal transition (EMT). [51)]

Until today, the correlation between lymph node metastasis and immunohistochemical markers has been investigated. However, no previous studies have validated the correlation between occult metastasis and immunohistochemical markers. Therefore, the correlation between occult metastasis and immunohistochemical markers were experimented in this study.

The aim of this study is as follows: (1) to analyze the incidence and clinical aspect of occult metastasis in patients who showed clinically N0 with tongue cancer (2) to find the relationship between clinicopathologic findings and occult metastasis (3) to find bio marker associated with occult metastasis by immunohistochemistry (4) to propose a useful diagnostic method for selecting the treatment.

II. Material and Methods

1. Patients

Patients who visited and underwent surgery in Seoul National

University Dental Hospital from 2000 to 2012 were included in this study. The patients were pathologically diagnosed with oral tongue cancer with no apparent cervical lymph node metastasis in pre-operative work up (Clinical examination, MRI, Ultrasonography, and PET). The patients were divided to 3 groups (Elective neck dissection group, Watchful waiting group, and Total group). The patients in the END group received glossectomy with END. Patients in the WW group received only glossectomy with watchful waiting. In the END group, after END, occult metastasis was investigated. In the WW group, neck recurrence was investigated in watchful waiting period. Lymph node metastasis of the Total group was a combined group consisting of patients with occult metastasis in the END group and neck recurrence in the WW group. Patients undergoing radiation therapy or chemotherapy before surgery were excluded in this study.

2. Clinical data collection

Age, sex, clinical manifestations, TNM stage, and survival analysis were reviewed through medical records and follow-up. Staging of primary site and cervical lymph nodes in oral tongue cancer was classified by American Joint Committee on Cancer (AJCC) 7th

Edition. The patients were divided into groups with an interval of 10 years of age from 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, 81 to 90, and 90 to 100 ages. Furthermore, the subjects were classified to male and female. Site of involvement was divided into lateral area and other region (floor of mouth, tongue base).

3. Histopathologic finding

For histopathologic review, 109 cases of H&E slides were reviewed in the Department of Oral Pathology at Seoul National University Dental Hospital from 2000 to 2012. Two oral pathologists reviewed H&E slides and identified the depth of invasion, differentiation, and T-stage. Pathologic report was reviewed. Sex, ages, area of tumor and site of occult metastasis were identified. The slides were examined under an optical microscope at a final magnification of x200.

4. Immunohistochemistry

4.1. Materials& Tissue microarray construction

Paraffin-embedded blocks of 41 cases of oral tongue cancer specimens from Department of Oral Pathology at Seoul National University Hospital from 2000 to 2012 were examined.

Two oral pathologists examined H&E-stained slides for this study. Slides representing cancer were selected from each case, and the area of tumor was circled. Paraffin blocks corresponding to the selected area were also circled with an oil marker pen. All Paraffin-embedded blocks were prepared for tissue microarray. (Figure I)

4-mm diameter needle was used for the core and the core was transferred to a recipient paraffin block. Tissue microarray is made by the method re-location of the tissue from paraffin blocks. Therefore, tissues from the blocks of multiple patients could be seen on one slide. The microarray blocks were sectioned to 3 ㎛, and was transferred to the glass. Afterwards, immunohistochemical staining was performed. Slides were stained with antibodies. These Slides were incubated in oven at 60℃ for 1 hour, deparaffinized with xylene, rehydrated by serial dilutions with alcohol (72℃ for 3 minutes, 3 times), and washed with tap water for 5 minutes.

4.2. Immunostaining (Table I)

IHC analysis of VEGF-c and COX-2 were performed using a Bond

polymer detection kit (Leica Microsystem Co., Ltd., Seoul, South Korea) with a monoclonal antibody against VEGF-c (1:500; Santa Cruz Biotechnologies, Inc., TX, USA), and COX-2 (1:300; Spring Bioscience, Inc., CA, USA). IHC analysis of c-Met was performed using an Ultraview detection kit (Ventana Medical Systems, Inc., AZ, USA) with monoclonal antibody against c-Met (RTU; Ventana Medical Systems, Inc., AZ, USA). IHC analysis of podoplanin was performed using Elite ABC kit (Vector Laboratories, Inc., CA, USA) with a monoclonal antibody against podoplanin (1:100; AngioBio, Inc., Ca, USA). IHC analysis of podoplanin was performed using Envision kit (Dako North America, Inc., CA, USA) with a polyclonal antibody against ROR1 (1:200; Santa Cruz Biotechnologies, Inc., TX, USA). (Table I)

For VEGF-c and COX-2, antigen retrieval was done at pH 6.0 (VEGF-c) and pH 9.0 (COX-2) using Epitope Retrieval 1 solution (Leica Microsystem Co., Ltd., Seoul, South Korea) for 20 minutes at 100° C. The reactions were then processed with peroxidase block solution for 5 minutes to quench endogenous peroxidase activity. Then, the slides were incubated with monoclonal antibodies for 15 minutes. Afterwards, the sections were incubated with bond polymer detection kit for 8 minutes. Slides were incubated for 10 minutes with 3, 3'-Diaminobenzidine (DAB) to visualize reaction.

Finally, the Slides were counterstained with Mayer's hematoxylin for 1 minute.

For c-Met, antigen retrieval was done at pH 8.4 using Cell conditioning 1 (Ventana Medical Systems, Inc., AZ, USA) for 60 minutes at 100° C. Then, the slides were incubated with a monoclonal antibodies for 32 minutes at 37° C. Afterwards, ultra wash was performed. Finally, the slides were counterstained with Mayer's hematoxylin for 4 minutes. After the counterstain, slides were incubated for 4 minutes in Bluing reagent.

For podoplanin, antigen retrieval was done at pH 6.0 with citrate buffer solution for 15 minutes at 100° C. The reactions were then processed with peroxidase block solution for 6 minutes to quench endogenous peroxidase activity. Slides were incubated for 25 minutes with Protein block (horse normal serum). Then, the slides were incubated with primary antibody for 60 minutes and with secondary antibody for 30 minutes. The slides were incubated for 30 minutes with ABC reagent. Afterwards, the slides were incubated for 3 minutes with DAB to visualize reaction. Finally, the slides were counterstained with Mayer's hematoxylin.

For ROR1, antigen retrieval was done at pH 9.0 with retrieval buffer (Dako North America, Inc., CA, USA) for overnight at 4° C. The reactions were treated with peroxidase block solution for 5

minutes to quench endogenous peroxidase activity. Then, the slides were incubated with primary antibody, followed by incubation with the labelled polymer, using two sequential 30-minutes incubation. Then, the slides were incubated for 10 minutes with DAB to visualize the reaction. Finally, the slides were counterstained with Mayer's hematoxylin.

4.3. Marker

4.3.1. VEGF-c

A final score for VEGF-C was defined as the sum of (a) and (b): (a) The intensity of the stain (0: negative; 1: weak; 2: moderate; 3: strong; 4: very strong) (b) The percentage of positive cancer cells (0: 0% of immunostained cells; 1: <25% of immunostained cells; 2: 25-50% of immunostained cells; 3: 50-75% of immunostained cells; 4: >75% of immunostained cells). The final score greater than 6 was considered as high expression.[61)]

4.3.2. C-Met

A final score for c-Met was defined as the sum of (a) and (b): (a) The intensity of the stain (0: none; 1: light yellow; 2: yellow brown;

3: brown) (b) The percentage of positive cancer cells (0: 0–5 % of immunostained cells; 1: 6–25 % of immunostained cells; 2: 26–50 % of immunostained cells; 3: 51–100 % of immunostained cells). The final score greater than 4 was considered as high expression.[62)]

4.3.3. COX-2

The staining were divided into the 4 groups (–: 0–4% of immunostained cells; +: 5–19% of immunostained cells; ++: 20–49% of immunostained cells; +++: 50–100% of immunostained cells). "++/+++" groups were considered as the high expression.[63)]

4.3.4. Podoplanin

The final score was calculated by multiplying staining intensity with percentage of positive cancer cells: (a) The intensity of the stain (0: no staining; 1: weak; 2: moderate; 3: strong) (b) The percentage of positive cancer cells (0: no staining; 1: 1–10% of immunostained cells; 2: 11–30% of immunostained cells; 3: 31–50% of immunostained cells; 4: 51–80% of immunostained cells; 5: >80% of immunostained cells).

The final score greater than 3 was considered as high expression.[64)]

4.3.5. ROR1

The staining types were divided into 3 groups (0: no staining; 1: low-level or low-to-moderate-level less than 50% of cancer cells; 2: moderate-level more than 50% of cancer cells or high level staining of the cancer cells). "2" group was considered as the high expression. "0, 1" groups were considered as the low expression.[54)]

5. Statistical analysis

Chi-square test and Fisher's exact test association were used to evaluate the association between lymph node metastasis and clinicopathologic factors. Overall survival rates were evaluated using Kaplan Meier method and values were compared by log-rank test. The correlation between immunohistochemical finding and lymph node metastasis was analyzed with the chi-square test, Fisher's exact test. Statistical tests were performed using SPSS software (version 21) (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

III. Result

1. Clinical and histological analysis

A total of patients with SCC were 109 patients. Distribution of clinical and pathological data (sex, age, T-stage, depth of invasion, differentiation, and tumor site) in the END group, WW group, and Total group are listed in Table II.

1.1. Lymph node metastasis

Among 71 patients who received END with glossectomy, occult metastasis was observed in 13 patients and neck recurrence was observed in 8 patients among the 38 patients who received glossectomy only. As a result, the incidence of occult metastasis in the END group was 18.3%. The incidence of neck recurrence in the WW group was 21.1% and in the total group 19.3%. (Figure II)

1.2. Sex and Age

81 of 109 patents were male and 28 female. The mean age was 54.4±15.4 years, ranging from 23 to 91. Occult metastasis and neck recurrence were most common between the ages of 40 to 60. (Table III, Figure III)

1.3. T-stage

In relation to the size of the primary tumor, occult metastasis was found in 2 among 32 patients of T1, 10 among 34 patients of T2, 0 among 1 patient of T3, and 1 among 4 patients of T4 in the END group. Neck recurrence was found in 6 among 30 patients of T1, 2 among 7 patients of T2, 0 among 0 patients of T3, and 0 among 1 patient of T4 in the WW group. (Table IV)

Patients in T2-4 group showed more occult metastasis than patients in T1 group, and this was statistically significant in the END group (P=0.017).

1.4. Site of primary tumor

In the END group, the incidence of occult metastasis varied depending on the primary site of the tumor. 8patients among 51 patients displaying primary tumor on the lateral surface, 4 among 18 patients on the floor of mouth, and 1 among 2 patients on the tongue base showed occult metastasis. In the WW group, neck recurrence was found in 7 patients among 35 patients on the lateral surface, 1 among 3 patients on the floor of mouth, 0 among 0 patients on the tongue base. Occult metastasis and neck recurrence were found from level I through to level IV. (Table IV)

1.5. Depth of invasion

To investigate the depth of invasion associated with occult metastasis or neck recurrence, patients were divided into 2 groups: those who have tumor with thickness of greater than or equal to 3 mm and tumor with thickness of less than 3 mm. The median depth of invasion in the END group was 0.77±0.56cm (Range 0.1−3.3). The median depth of invasion in the WW group was 0.46 ± 0.34cm (Range 0.1−1.5).

The group with thickness of greater than or equal to 3 mm showed more incidence of lymph node metastasis than the group with thickness of less than 3 mm. Depth of invasion greater than or equal to 3 mm in the Total group was statistically correlated with occult metastasis (P=0.022).(Table IV)

1.6. Differentiation

Occult metastasis was found in 11 patients among 61 patients with well differentiation, 2 among 8 patients with moderate differentiation, and 0 among 2 patients with poor differentiation in the END group. Neck recurrence was found in 7 patients among 36 patients with well differentiation, 1 among 2 patients with moderate differentiation, and 0 among 0 patients with poor differentiation in

the WW group. Moderate/poor differentiation group showed more incidence of lymph node metastasis than well differentiation group. But all values were not statistically significant. (Table IV)

1.7. Neck dissection

END group was treated with selective neck dissection. WW group was treated with RND and FND after neck recurrence. Therefore, WW group was treated more aggressively, after neck recurrence, than the END group. (Table V)

1.8. Site of occult metastasis and neck recurrence

In the END group, occult metastasis was found in 4 patients at level I, 2 patients at level II, 4 patients at level III, and 1 patient at level IV. Two patients displayed occult metastasis at multiple levels (level II, III).

In the WW group, LNM was found in 2 patients at level I, 2 patients at level II, and 1 patient at level III. Three patients displayed LNM at multiple levels (level II, III: 2patients, level I, III, IV: 1patient). (Table VI)

2. Survival analysis

The 3- and 5-year overall survival rates of the WW group were 88.4% and 84.3% respectively, and the rates of the END group were 75.8% and 71.9%, respectively. Patients in the WW group showed better survival rate than patients in the END group, although this was not statistically significant (p=0.068). (Figure IV)

The 3- and 5-year overall survival rates of the pN0 patients in the END group were 80.9% and 80.9% respectively, and the rates of the pN(+) group were 51.9% and 31.2%, respectively. This value was statistically significant (p=0.001). Patients in the pN0 group showed better survival rate than patients in the pN(+) group. (Figure V)

The patients in the negative neck recurrence group showed better survival rate compared to the patients in the positive neck recurrence group. The 3- and 5-year overall survival rates in the negative neck recurrence group were 92.3% and 86.8% respectively, and in the positive neck recurrence group the rates were 75.0% and 75.0%, respectively. However, the OS according to neck recurrence was not statistically significant (p=0.331). (Figure VI)

OS according to differentiation was not statistically significant (p=0.061). Nonetheless, the patients in the well differentiation

group showed better survival rate compared to the patients in the moderate/poor differentiation group. The 3- and 5-years overall survival rates in well differentiation group were 83.1% and 79.8% respectively, and the rates in the moderate/poor differentiation group were 58.3% and 48.6%, respectively. (Figure VII)

3. Immunohistochemical study

Immunohistochemical reactivity for VEGF-c, c-met, Cox-2, podoplanin, ROR1 are summarized in Table.VII

3.1. VEFG-c

Immunostaining for VEGF-c was detected in the cytoplasm. Images of immunohistochemical staining for VEFG-c are shown in Figure VIII.

Positive VEGF-c expression was significantly correlated with occult metastasis in the total group (p=0.043). However, positive VEGF-c expression had no significant relationship with the occult metastasis in the END group (p=0.417) and the neck recurrence in the WW group (p=0.145).

3.2. c-Met

Immunostaining for c-Met was detected in the cytoplasm and the cytoplasmic membrane. Images of immunohistochemical staining for c-Met are shown in Figure IX.

Positive c-Met expression was significantly correlated with occult metastasis in the Total group. (p=0.009) However, positive c-Met expression had no significant relationship with the occult metastasis in the END group (p=0.127) and the neck recurrence in the WW group (p=0.092).

3.3. COX-2

Immunostaining for COX-2 was detected in the cytoplasm. Images of immunohistochemical staining for COX-2 are shown in Figure X.

All values had no statistical significance with lymph node metastasis in all group. The number of high expression was observed more than the number of low expression in all groups.

3.4. Podoplanin

Podoplanin was not stained in all tissues. Image of immunohistochemical staining for podoplanin is was shown in Figure XI.

3.5. ROR1

Immunostaining for ROR1 was detected in the cytoplasm and nucleus of cancer cells. Images of immunohistochemical staining for ROR1 are shown in Figure XII.

Positive ROR1 expression was significantly correlated with occult metastasis in both the Total group (p=0.003) and the END group (p=0.013). Positive ROR1 expression had no significant relationship with neck recurrence in the WW group. (p=0.188)

IV. Discussion

1. Occult metastasis

Clinically N0 means that LNM is not diagnosed clinically or radiologically. Occult metastasis means that LNM was not diagnosed clinically or radiologically, but LNM is detected on biopsy. 20-50% of occult metastasis has been found in oral tongue cancer patients. (Table VIII) In this study, the incidence of occult metastasis in the END group, neck recurrence in the WW group, and occult metastasis in the Total group were 18.3%, 21.1%, 19.3%. (Figure II)

The incidence of the occult metastasis of oral tongue cancer has been reported variously. Jones *et al.* reported that the incidence of

occult metastasis was 29% in patients with OSCC and that the main site of the primary tumor was lateral area of the tongue and floor of mouth.[65] The incidence of the occult metastasis in patients with OSCC of cT1N0 was 13-33%. The incidence of the occult metastasis in patients with OSCC of cT2N0 was 37-53%.[11,66-69] LNM has been considered a significant prognostic factor in oral cancers.[70-72] The survival rate of Patients with OSCC is reduced by 50% if there is a lymph node metastasis.[73,74] Early detection in patients with occult metastasis could improve survival rate.

2. Skip metastasis

Skip metastasis may occur occasionally. Skip metastasis is found at level III or Level IV without metastasis at level I, II or Level I, II, III.

Byers *et al.* reported that skip metastasis was 15.8%.[75] Lim *et al.* reported that level IV metastasis was 2% in T1-3 N0 oral tongue cancer. If there is a suspected lymph node at level II or level III, Level IV lymph nodes should be removed.[76]

Data in this study showed that the incidence of skip metastasis at level III or level IV was 38% (5 patients out of the 13 patients) in the END group. The incidence of skip metastasis was 12.5% (1 patient of the 8 patients) in the WW group. In this study, a high

incidence rate of skip metastasis was observed. (Table VI)

3. Depth of invasion

Several papers have examined the relationship between depth of invasion and LNM. Spiro *et al.* and Brown *et al.* recommended END when Tumor thickness exceeds 2 mm in patients with oral cancer.[77,78] Mohit-Tabatabai *et al.* recommended END when Tumor thickness exceeds 1.5 mm in patients with N0 oral cancer.[79] Rasgon *et al.* reported that depth of invasion greater than 5 mm showed an high incidence of LNM.[80] Spiro *et al.* suggested END when Tumor thickness exceeds 1.5 mm.[77]

This study was that the group with thickness of greater than or equal to 3 mm showed more incidence of LNM than the group with thickness of less than 3 mm. It is thought that the patients with thickness of greater than or equal to 3 mm should be treated with END.

4. T stage

Some authors reported that there was a correlation between lymph node metastasis and T stage[9,77,79,81-83] while others reported no correlation.[80,84-88] Result of this study was that Patients in T2-4 group showed more occult metastasis than Patients in T1 group.

It is thought that the patients with T2, 3, 4 should be treated with END.

5. Differentiation

Okada *et al.* reported a significant correlation between histological grade and the incidence of lymph node metastasis in oral SCC.[89] Similarly, Mendelson *et al.*, Umeda *et al.*, and Frierson *et al.* reported that patients with poor differentiation were more incidence of lymph node metastasis than patients with well differentiation.[81,87,90]

This study found that differentiation had no significant correlation with lymph node metastasis. However patients with well differentiation showed more incidence of lymph node metastasis than patients with moderate/poor differentiation. (Table IV)

6. Survival analysis

Some studies failed to gain statistically significant differences about survival rate between the END group and the WW group[91-94] while other studies showed survival benefit in the END group.[66,95,96]

Hiratsuka *et al.* reported that 5-year survival rates of patient group with occult metastasis and without occult metastasis were 94%

and 51%, respectively.[97] Keski *et al.* reported that depth of invasion, T stage, N stage, and histological differentiation were significantly correlated with survival rate in patients with early oral tongue cancer.[98] O-charoenrat *et al.* reported that depth of invasion greater than 5mm was significantly correlated with poor survival rate. However, T stage, N stage, invasive form, and histological differentiation were not significantly correlated with survival rate.[99]

In this study, Patients with the pN0 showed better survival rate than patients in the pN (+) group in the END group. (Figure V) However, the relevance with other factors (treatment modality, T stage, differentiation) was not shown. The existence of LNM is the most important prognostic factor for survival of head and neck cancer patients. Patients in the WW group showed better survival rate than patients in the END group (Figure IV). It is thought that the number of T1 patients was more than the number of T2 patients in WW group, but the number of T2 patients was more than the number of T1 patients in END group.

7. Treatment modality

There were several retrospective reports on both advocating

group[13,66,81,94,100-102] and opposing group[85,103-107] about END. Some studies showed increased survival benefit in the END group with oral cancer.[66,96,102,108] However, other studies did not show statistically significant differences in survival rates between the END group and the WW group.[67,91,92,94,102] The choice of treatment is often difficult and controversial. In this study, the result did not show statistically significant differences in survival rates between the END group and the WW group. However, patients in the WW group showed better survival rate than patients in the END group. Therefore, it is thought that END has no benefit about survival rate.

7.1. Elective neck dissection

If LNM is not found, lymph nodes may be severely metastasized and extracapsular metastasis may be occurred. After END, the occult metastasis can be detected pathologically. END can provide an opportunity of high survival rate via post-operative radiation therapy.

Franceschi *et al.* reported that LNM with neck dissection can be found early in tongue cancer patients. Because additional radiation treatment is possible, Survival rate of the END group is higher than survival rate of the WW group.[85] If patients with occult metastasis are not treated by neck dissection, poor treatment outcome and

poor survival rate would be shown.[109] However, patients of the WW group showed better survival rate than patients of the END group in this study.

7.2. Watchful waiting

Authors advocating watchful waiting, recommended therapeutic neck dissection due to complications of prophylactic neck dissection.[110] Vandenbrouck *et al.* reported that Prophylactic neck dissection had no advantage compared to therapeutic neck dissection. Therefore neck dissection cause prolonged hospitalization and increased mortality in these patients.

8. Closed follow up

Some investigators insisted that routine END was avoided because of unnecessary morbidity and doubtful survival benefit. However, closed follow up is the most important factor in the WW group. Because, if the metastatic lymph nodes during follow up are found to be smaller in size, there will be less probability of extracapsular spread. Thus, they will be salvaged successfully.

Myers *et al.* reported that 5-year overall survival rates of pathologically negative, pathologically positive without

extracapsular spread(ECS), and pathologically positive with ECS were 73%, 50%, and 30% in patients with oral tongue cancer.[111)]

Most of all, Close follow up is essential to obtain similar results between the WW group and the END group.[112)]

9. Immunohistochemical finding

In this study, it was examined whether the expression of the following 5 markers are correlated with occult metastasis and whether they are useful marker for detection of occult metastasis. (Table VII).

9.1. VEGF-c

VEGF-c expression is associated with lymphatic invasion and LNM. VEGF-c promotes lymphangiogenesis and enhances invasion *via* loosening of lymphatic endothelial cells.[113)] High expression of VEGF-c correlates with the LNM in human thyroid, lung, prostate, gastric, colorectal, breast cancer, cervical cancer, and melanoma.[20-22,114)]

In this study, positive VEGF-c expression was significantly correlated with occult metastasis in the total group (p=0.043). This marker is thought to be expressed in early stage of lymph node

metastasis proceeds.

9.2. c-Met

c-Met is expressed in epithelial cells of many organs, including the prostate, pancreas, liver, muscle, bone marrow and kidney during both embryogenesis and adulthood.[115] c-Met regulates cellular processes, cell function, and tissue homeostasis in mammalian development.[23] The activation of c-Met increases the cancer cell proliferation, motility, invasion, and survival rate.[26,116,117] c-Met expression level was significantly correlated with LNM.[118] In oral squamous cell carcinoma, several studies proved that overexpression of c-Met was a considerable pathologic parameter for metastasis.[119,120] Similarly, Dan et al found that expression level of c-Met was correlated with positive lymph node, advanced clinical stage, and recurrence. High expression level of c-Met was associated with poor 5-year overall survival rate and poor disease-free survival rate.[121]

In this study, Positive expression of c-Met was significantly correlated with occult metastasis in the total group ($p=0.009$). This marker is thought to be expressed in early stage of lymph node metastasis. c-Met might contribute to occult metastatic process, and c-Met might facilitate the invasion of cancer cells into the

lymphatic vessels.

9.3. COX-2

COX-2 metabolizes arachidonic acid and activates cell membrane phospholipid-derived inflammation. Also, COX-2 increases in various cancer cells. High level of COX-2 expression is correlated with tumor growth, metastasis, apoptosis, angiogenesis, and suppression of antitumor immunity.[32,122-128] Several studies have demonstrated a correlation between COX-2 expression and lymph node metastasis in gastric, lung, breast, prostate cancer and oral squamous cell carcinoma.[38,39,129,130] Several studies have demonstrated possibility as chemopreventive agents in oral squamous cell carcinoma.[131,132] Tumor was diminished when animals were treated with inhibitors of COX-2 in animal model.[133,134] In this study, all values were not statistically significant in all groups. High expression was more frequently observed than low expression in all groups.

9.4. Podoplanin

Podoplanin is a lymphatic endothelial marker which can be used to differentiate lymphatic vessels from blood vessels. In oral lesions, it is expressed in leukoplakia, premalignant lesion. Also, it can be

served as a marker for predicting the risk of oral cancer.[135] Several studies have showed a role of podoplanin in metastasis and invasion.[136,137] Podoplanin-expressing cells were found at invasion site in OSCC.[138] Huber *et al.* reported that Podoplanin expression correlates with sentinel lymph node metastasis in early squamous cell carcinomas of the oral cavity and oropharynx.[64] However, podoplanin was not stained in all tissues in this study.

9.5. ROR1

Receptor tyrosine kinases have important functions in proliferation, angiogenesis, cell differentiation, and migration.[139,140] ROR1 is one of the ROR families (ROR1, ROR2). RORs consist of two extracellular cysteine rich domains and one transmembrane domain. ROR1 is a type-I membrane protein that is expressed during embryogenesis, and it is important for the morphogenesis of many organs.[60] While ROR1 expression is detected during normal embryonic and fetal development, it is not detected in most mature tissues.

Although the exact function of ROR1 is not found, many studies revealed that ROR1 is associated with progression, development and metastasis of various human cancers. ROR1-mediated signaling has been shown in various cell lines. Wnt5a (ligand of ROR1)

activates NF-kB in HEK293.[141] Wnt5a involves in the ROR1-dependent signaling pathway enhancing cancer cell growth.[54] In adenocarcinoma cell lines, ROR1 can phosphorylate c-SRC. The EGF-induced signaling is magnified through interaction of the FZD and EGFR.[139] In gastric carcinoma and lung carcinoma cell lines, ROR1 is phosphorylated by MET; the silencing of ROR1 decreases cell growth.[57] In breast cancer cells, ROR1 expression is significant correlated with EMT genes. Silencing of ROR1 reduces EMT genes (ZEB1, SNAI1, SNAI2 and vimentin).[56] Treatment with antibody of ROR1 can decrease cancer progression and metastasis.[56]

In this study, Positive ROR1 expression was significantly correlated with occult metastasis in the Total group (p=0.003), in the END group (p=0.013). ROR1 is thought to be expressed in early stage of LNM.

V. Conclusion

Patients with thickness of greater than or equal to 3 mm and with more advanced T stage showed more incidence of LNM. Patients in pN0 group showed better survival rate compared to patients pN(+) group. VEGF-c, c-Met, ROR1 had statistically significant

correlation with occult metastasis. VEGF-c, c-Met, ROR1 are thought to be expressed in early stage of LNM.

This study showed a relatively high incidence of occult metastasis and even showed skip metastasis in tongue cancer patients with N0 neck. By considering clinical, histological, and immunohistochemical factors, surgeon can determine whether to treat by END or WW. First of all, close follow up is important to obtain similar results between the WW group and the END group.

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Tables

Table. I. Antibody used & source in this study.

Antibody (Clone)	Clonality	Dilution	Retrieval buffer	Detection kit	Source (Cat.No.)
VEGF-c	Mouse monoclonal	1:500	pH6.0 Bond epitope retrieval solution1	Bond polymer detection kit (Leica)	Santa cruz (SC-7269)
c-Met	Rabbit monoclonal	RTU	pH 8.4 cell conditioning1	Ultraview detection kit (ventana)	Ventana (790-4430)
COX-2	Rabbit monoclonal	1:300	pH9.0 Bond epitope retrieval solution 2	Bond polymer detection kit (Leica)	Spring bioscience (M3212)
Podoplanin	Mouse monoclonal	1:100	pH6.0 citrate buffer	Elite ABC kit (Vector)	Abcam (ab63371)
ROR1	Rabbit polyclonal	1:200	pH9.0 Retrieval buffer (Dako)	Envision kit (Dako)	Santa cruz (P-288)

Cat. No. ; Category number

Table. II. Clinico-pathologic characteristics of patients.

(Unt :%)

		Total	END	WW
Sex	M	81(74.3)	58(81.7)	23(60.5)
	F	28(25.7)	13(18.3)	15(39.5)
Age	≥50	69(63.3)	44(62)	25(65.8)
	<50	40(36.7)	27(38)	13(34.2)
T stage #	I	62(56.7)	32(45.1)	30(78.9)
	II	41(37.6)	34(47.9)	7(18.4)
	III	1(0.9)	1(1.4)	0(0)
	IV	5(4.8)	4(5.6)	1(2.7)
	I	62(56.9)	32(45)	30(79)
	II-IV	47(43.1)	39(55)	8(21)
Depth of invasion	≥3	82(75.2)	59(83.1)	23(60.5)
	<3	27(24.8)	12(16.9)	15(39.5)
Differentiation	Well	97(89.0)	61(85.9)	36(94.7)
	Moderate/Poor	12(11.0)	10(14.1)	2(5.3)
Area	Lateral	86(78.9)	51(71.8)	35(92.1)
	Other region (FOM, Base)	23(21.1)	20(28.2)	3(7.9)

END, elective neck dissection; WW, watchful waiting; M, male; F, female; FOM, floor of mouth; Is, carcinoma in situ; #Staging by AJCC 7th edition

Table. III. Occult metastasis or neck recurrence on basic of age

patient.

(Unt :%)

Age	Total (NR+OM)	Occult Metastasis (END)	Neck Recurrence (WW)
20	0/8 (0)	0/6 (0)	0/2 (0)
30	1/11 (9.1)	1/8 (12.5)	0/3 (0)
40	4/21 (19)	3/13 (23.1)	1/8 (12.5)
50	8/27 (29.6)	3/17 (17.6)	5/11 (45.5)
60	5/21 (23.8)	5/17 (29.4)	0/4 (0)
70	2/16 (12.5)	1/10 (10)	1/6 (16.6)
80	1/3 (33.3)	0/0 (0)	1/3 (33.3)
90	0/1 (0)	0/0 (0)	0/1 (0)

NR, neck recurrence: OM, Occult metastasis; END, elective neck dissection: WW, Watchful waiting

Table. IV. Correlation of clinico-pathologic parameters versus occult

metastasis and neck recurrence.

(Unt :%)

		Total (NR+OM)	*P*	END (OM)	*P*	WW (NR)	*P*
Sex	M	17/81 (21)	0.438	13/58 (22.4)	0.107	4/23 (17.4)	0.687
	F	4/28 (14.3)		0/13 (0.0)		4/15 (26.6)	
Age	≥50	16/69 (23.2)	0.173	9/44 (20.5)	0.754	7/25 (28.0)	0.222
	<50	5/40 (12.5)		4/27 (14.8)		1/13 (7.7)	
T stage #	1	8/62 (12.9)	0.053	2/32 (6.3)	0.017*	6/30 (20.0)	1.000
	2−4	13/47 (27.7)		11/39 (28.2)		2/8 (25.0)	
Depth of invasion	≥3	19/82 (23.2)	0.022*	13/59 (22.0)	0.106	6/23 (24.0)	0.216
	<3	2/27 (7.4)		0/12 (0.0)		2/15 (13.3)	
Differentiation	Well	18/97 (18.6)	0.698	11/61 (18.0)	1.000	7/36 (19.4)	0.381
	Moderate /Poor	3/12 (25)		2/10 (20.0)		1/2 (50)	
Area	Lateral	15/86 (17.4)	0.363	8/51 (15.7)	0.496	7/35 (20.0)	0.381
	Other region (FOM, Base)	6/23 (26.1)		5/20 (25.0)		1/3 (33.3)	

NR, neck recurrence; OM, occult metastasis; END, elective neck dissection; WW, watchful waiting; M, male; F, female; FOM, floor of mouth; # Staging by AJCC 7th edition
*Statistically significant (*P*<0.05)

Table. V. Neck management according to primary treatment.

	WW (No.)	END (No.)
Neck management	RND (6)	SND123(54)
	FND (2)	Both SND123(5)
		SND12 (4)
		SND1234 (3)
		SND 1235 (2)
		SND123 SND1 (2)
		SND1 SND12 (1)

WW, watchful waiting; END, Elective neck dissection; RND, Radical Neck Dissection; FND, Functional neck dissection; SND, Selective Neck dissection.

Table. VI. Site of occult metastasis or neck recurrence.

Level	Total Pt No. (LN No.)	Occult metastasis Pt No. (LN No.)	Neck recurrence Pt No. (LN No.)
Ⅰ	5(1), 1(2)	3(1), 1(2)	2(1)
Ⅱ	1(1), 3(2)	1(1), 1(2)	2(2)
Ⅲ	4(1), 1(3)	4(1)	1(3)
Ⅳ	1(1)	1(1)	0
Ⅱ&Ⅲ	4(1)	2(1)	2(1)
Ⅰ &Ⅲ & Ⅳ	1(5) Ⅰ(2)&Ⅲ(1) & Ⅳ(2)	0	1(5) Ⅰ(2)&Ⅲ(1) & Ⅳ(2)

LN No., Lymph node Number; Pt No., patients number; Staging by AJCC 7th edition

Table. VII. Immunohistochemical finding.

(Unt :%)

		Total (OM+NR)		P^a	END (OM)		P^a	WW (NR)		P^a
		Negative	Positive		Negative	Positive		Negative	Positive	
VEGF-c	Low	13 (65)	7 (33.3)	0.043*	5 (50)	4 (30.8)	0.417	8 (80)	3 (27.3)	0.145
	High	7 (35)	14 (66.7)		5 (50)	9 (69.2)		2 (20)	5 (71.4)	
c-Met	Low	8 (40)	1 (4.8)	0.009*	4 (40)	1 (7.7)	0.127	4 (40)	0 (0)	0.092
	High	12 (60)	20 (95.2)		6 (60)	12 (92.3)		6 (60)	8 (100)	
COX-2	Low	6 (30)	3 (14.3)	0.277	3 (30)	2 (15.4)	0.618	3 (27.3)	1 (14.3)	1.000
	High	14 (70)	18 (85.7)		7 (70)	11 (84.6)		8 (72.7)	6 (85.7)	
Podoplanin	Low	-	-	-	-	-	-	-	-	-
	High	-	-		-	-		-	-	
ROR1	Low	13 (65)	4 (19)	0.003*	7 (70)	2 (15.4)	0.013*	6 (60)	2 (25)	0.188
	High	7 (35)	17 (81)		3 (30)	11 (84.6)		4 (40)	6 (75)	

NR, neck recurrence: OM, occult metastasis: END, elective neck dissection: WW, watchful waiting
*Statistically significant (P<0.05) L, low: H, high.
[a]By chi-square, Fisher' s exact test

Table. VIII. Incidence of occult metastasis or neck recurrence in the

tongue cancer.

Author	Date	No.	T Stage	Site	Total N (%)	OM N (%)	NR N (%)
Lee et al.	1972	94	T1-T2	ant 2/3	22/94 (23)	5/13 (38.4)	17/81 (21)
Mendelson et al.	1976	295	T1-T3	ant 2/3	58/295 (20)	26/126 (20.6)	32/169 (18.9)
Vandenbrouck et al.	1980	75	T1-T3	ant 2/3 & FOM	36/75 (48)	19/39 (48.7)	17/36 (47.2)
Teichgraeber et al.	1984	48	T1-T2	ant 2/3	17/48 (35)	8/20 (40)	9/28 (32.1)
Spiro et al.	1986	92	T1-T3	ant 2/3 & FOM	25/92 (27)	8/29 (27.6)	17/63 (27)
Cunningham et al.	1986	23	T1-T2	ant 2/3	8/23 (35)	1/7 (14.3)	7/16 (43.7)
Fakih et al.	1989	70	T1-T2	ant 2/3	33/70 (47)	10/30 (33.3)	23/40 (57.5)
Ho et al.	1992	28	T1-T2	ant 2/3	10/24 (42)	0 (0)	10/24 (4 excluded) (42)
Franceschi et al.	1993	211	T1-T2	ant 2/3	65/211 (31)	26/63 (41.3)	39/148 (26.3)
Our result	2014	112	T0-T4	Tongue	21/109 (19.3)	13/71 (18.3)	8/38 (21.1)

NR, neck recurrence: OM, Occult metastasis: N, number

Figure Legends

Figures

Fig. I. Tissue microarray (TMA) for immunohistochemistry.

(http://www.tissue-

array.com/zoom2.php?img=/upload/gallery/22837/4_slides.jpg)

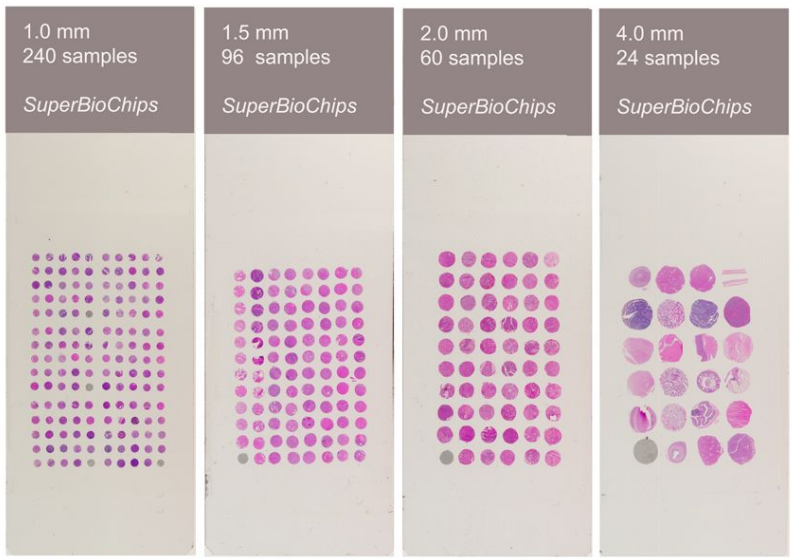


Fig. II. Incidence of occult metastasis and neck recurrence.

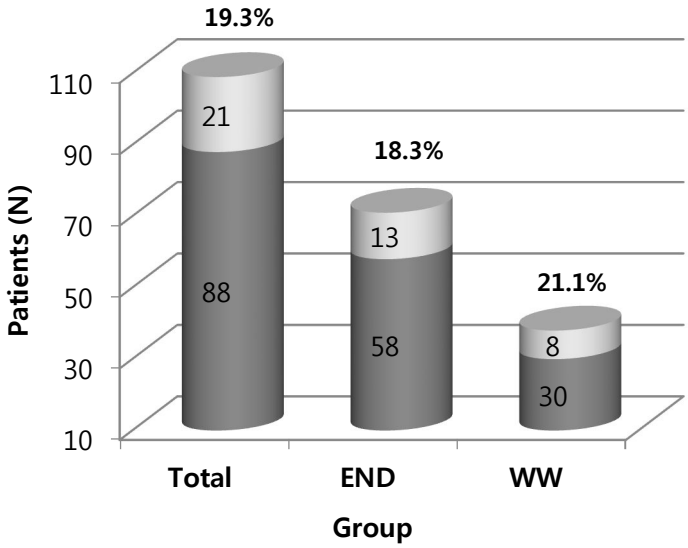


Fig. III. Occult metastasis or neck recurrence on basic of age patient.

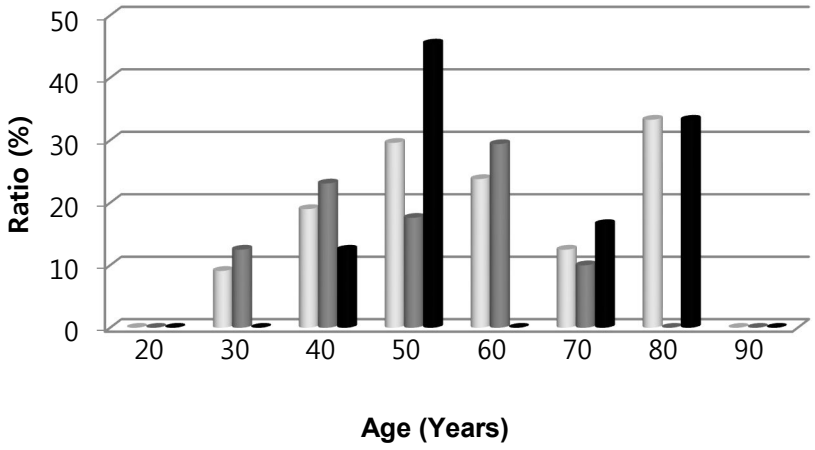

50
40
30
20
10
0
Ratio (%)
20
30
40
50
60
70
80
90
Age (Years)

Fig. IV. Overall survival according to different treatment (Kaplan-Meier curves with univariate analysis: log-rank).

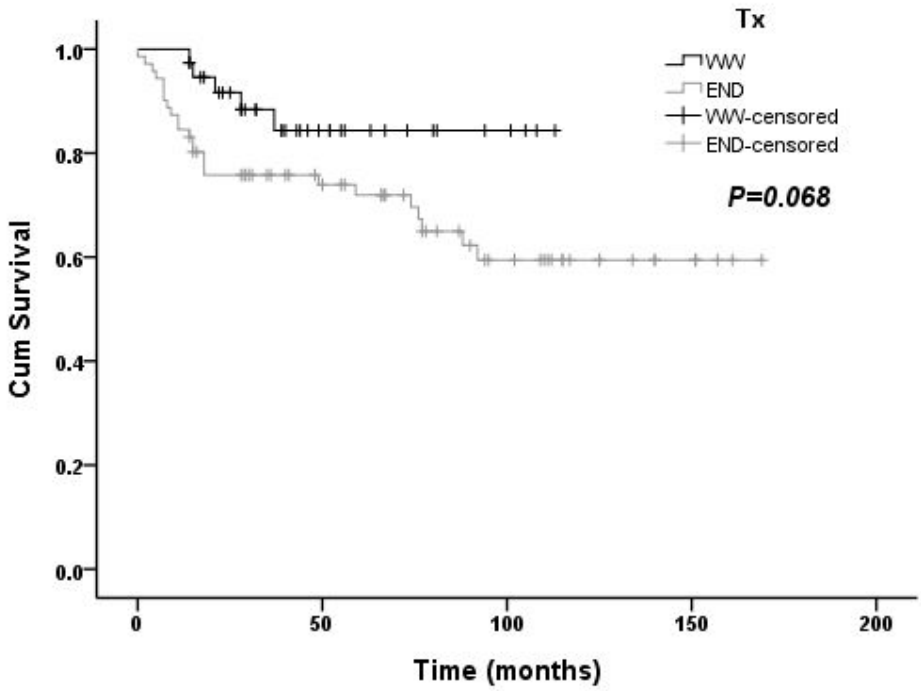


Fig. V. Overall survival according to occult metastasis in END group (Kaplan-Meier curves with univariate analysis: log-rank).

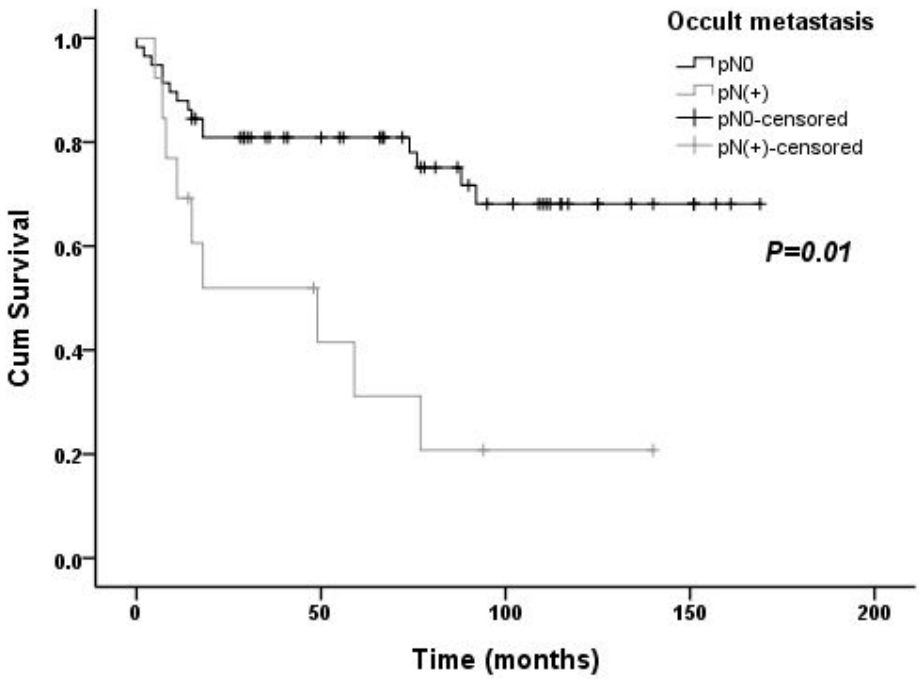


Fig.VI. Overall survival according to neck recurrence in WW group (Kaplan-Meier curves with univariate analyses: log-rank).

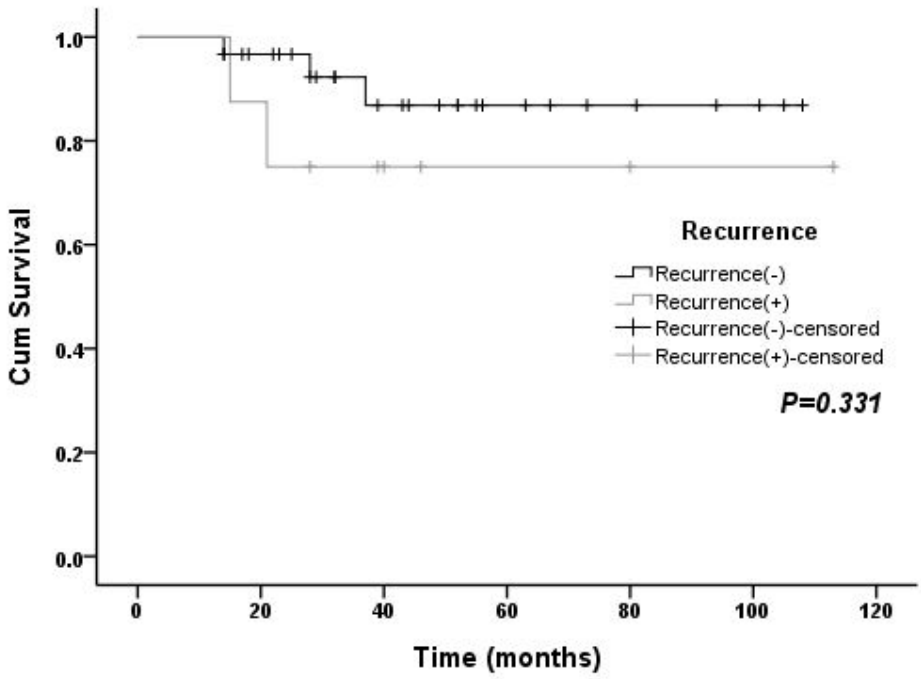


Fig. VII. Overall survival according to differentiation in total group (Kaplan-Meier curves with univariate analyses: log-rank).

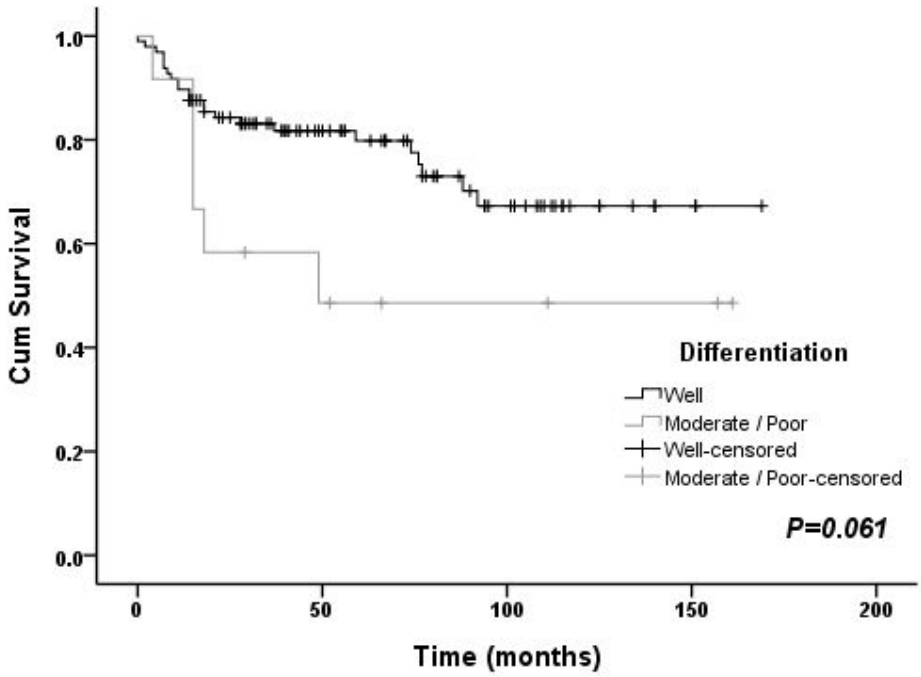


Fig. VIII. Expression of VEGF-c (x200 magnification).

(A) Low expression, (B) High expression

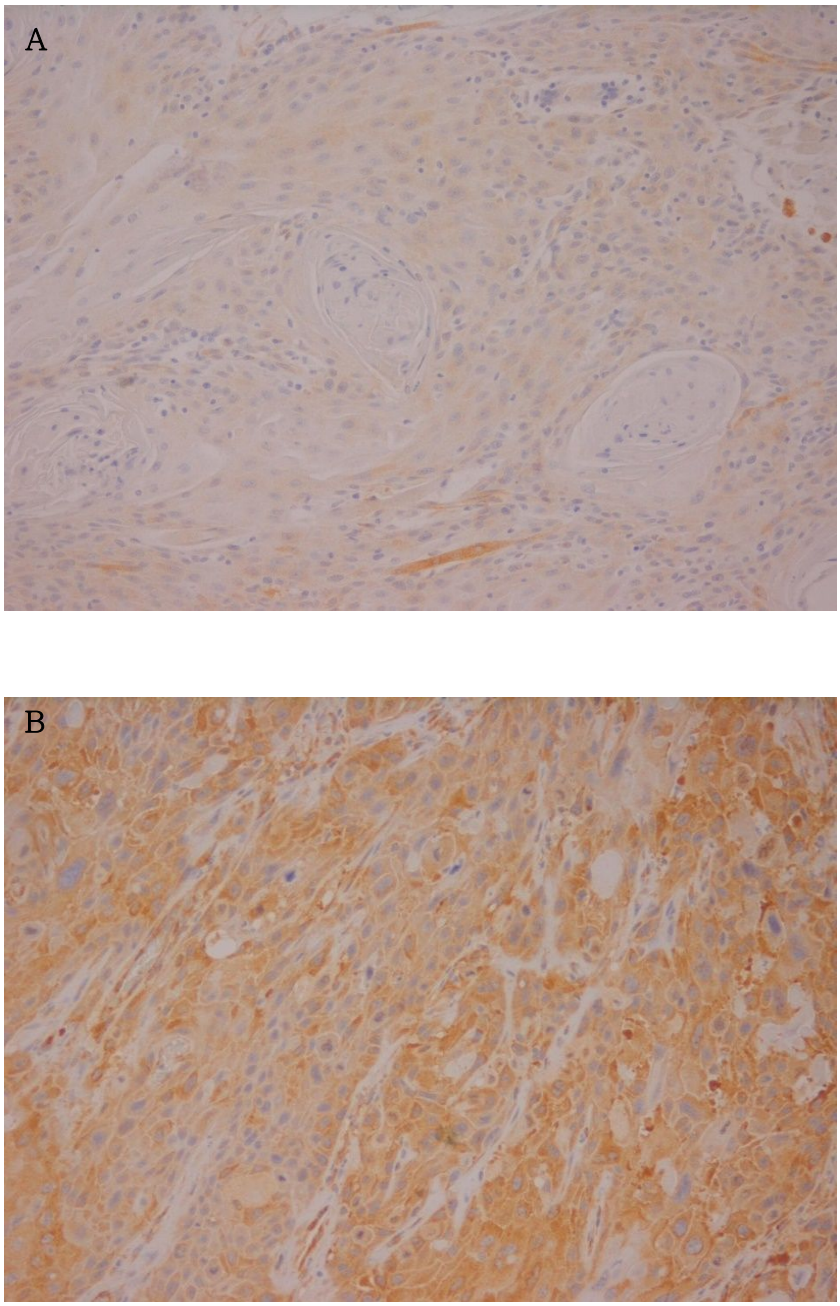


Fig. IX. Expression of c-Met (x200 magnification).

(A) Low expression, (B) High expression

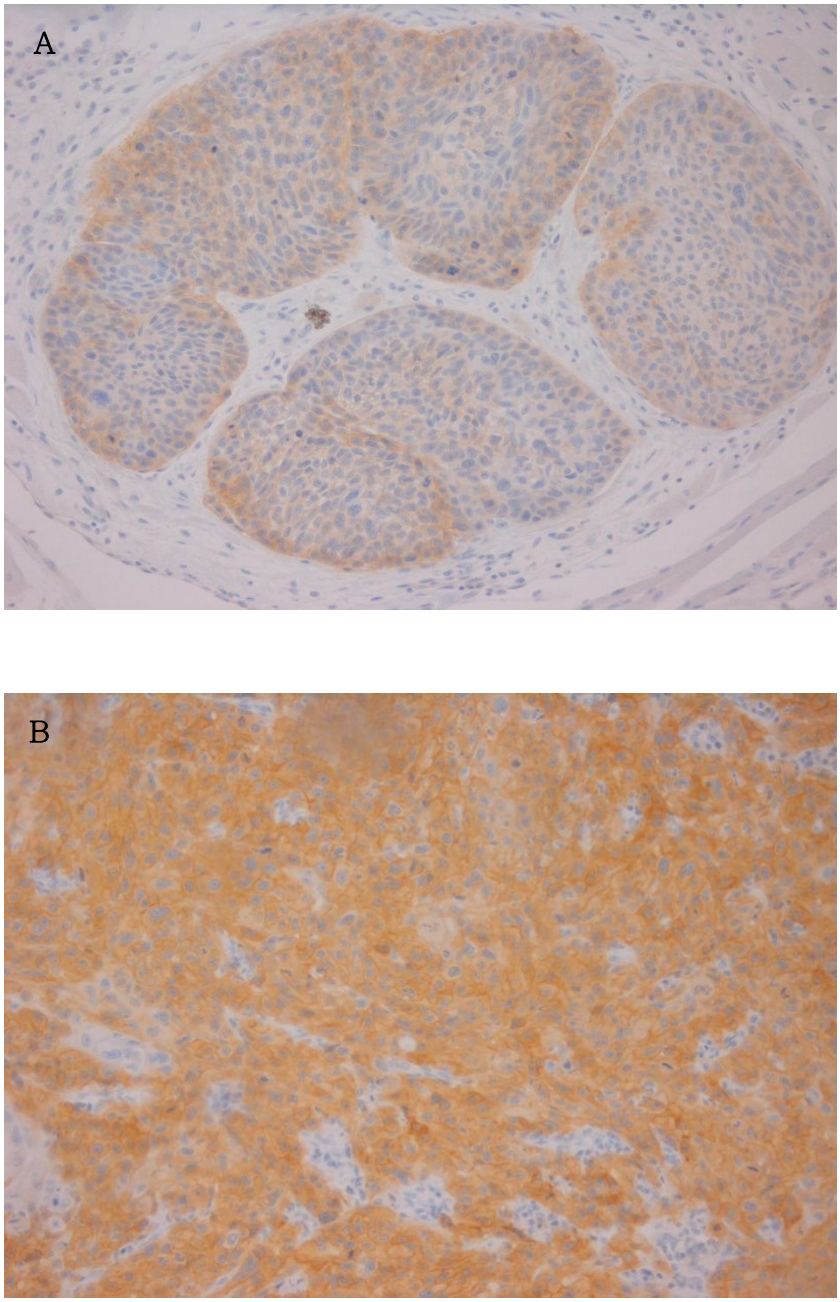


Fig. X. Expression of COX-2 (x200 magnification).

(A) Low expression, (B) High expression

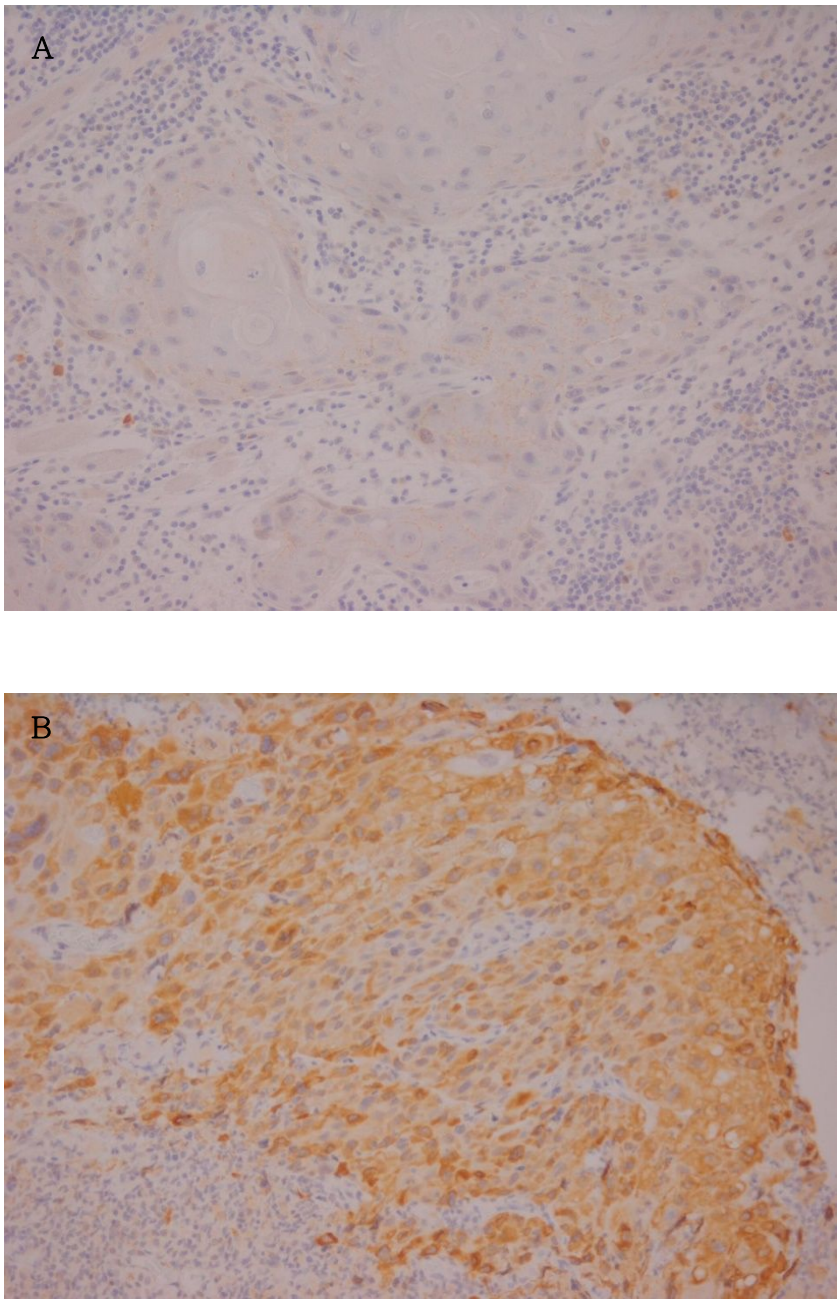


Fig. XI. Expression of podoplanin (x200 magnification).

(A) Negative

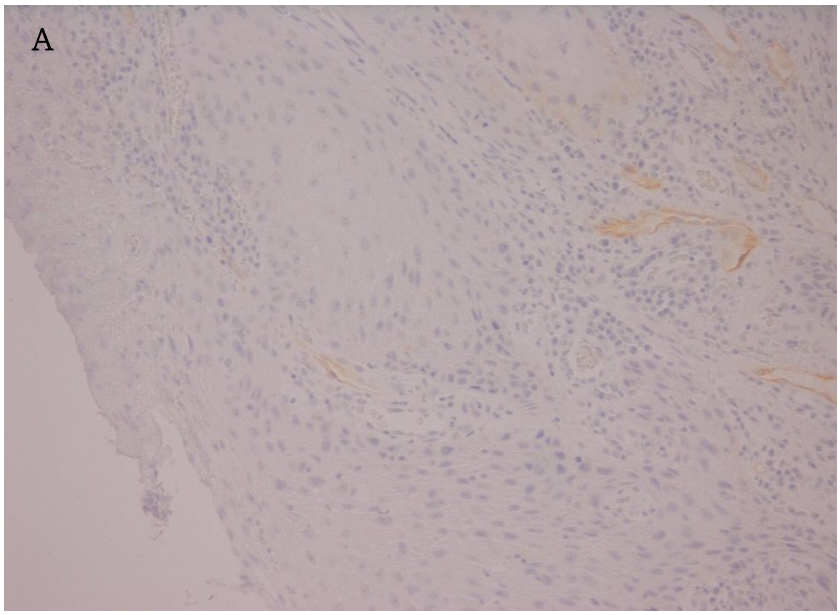
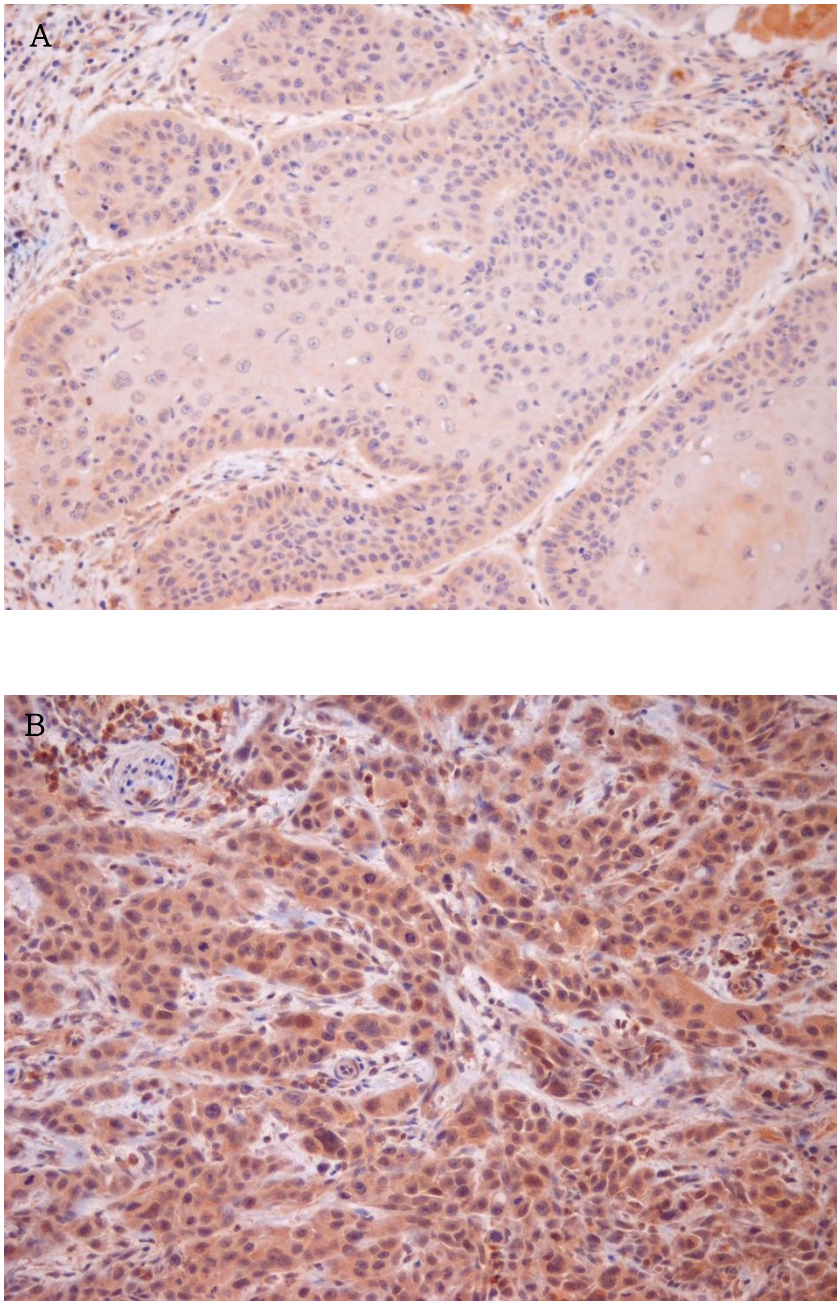


Fig. XII. Expression of ROR1 (x200 magnification).

(A) Low expression, (B) High expression



– 국문초록 –

임상적으로 림프절 전이가 없는 설암 환자에서 잠재성 림프절 전이에 대한 연구

신 정 현

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연구목적

본 연구의 목적은 술 전에 임상적 방사선학적으로 림프절 전이가 없는 설암 환자에서 술 후 조직검사 상 잠재성 림프절 전이 (occult metastasis)의 발생 빈도와 양상을 조사하고, 임상적 조직학적 인자와 비교하여 관련을 찾는데 있다. 또한 면역조직화학염색법을 이용하여 잠재성 림프절 전이와 표시자들과의 관련성을 발견하여, 치료 방법의 선택 시 유용한 진단 방법을 제시하고자 한다.

연구방법

서울대학교 치과병원 구강악안면외과에서 2001년부터 2012년까지 설암으로 진단된 후 술 전 임상적 방사선학적 검사상(임상적 검사, 자기공

명영상, 초음파 검사, 양전자 단층촬영) 경부 림프절 전이가 나타나지 않은 109명을 대상으로 하였다. 환자는 3군 (예방적 경부청소술군, 추적관찰군, 전체군)으로 분류 하였다. 예방적 경부청소술군의 환자들은 예방적 경부청소술과 혀 절제술 받았다. 추적관찰군의 환자들은 혀 절제술 만을 받았다. 예방적 경부청수술군은 수술 후 잠재성 림프절 전이를 조사 하였고, 추적관찰군은 추적관찰 기간 중에 경부 재발을 조사 하였다. 전체 그룹의 림프절 전이는 예방적 경부청소술군의 잠재성 림프절 전이와 추적관찰군의 경부재발을 합한 것으로 조사하였다. 술 후 조직검사에서 림프절 전이의 발생 빈도와 전이 위치 (경부 레벨), 치료방법, 술 후 재발 등을 조사하였다. 종물 절제술과 경부청소술을 동시에 시행한 군, 종물 절제술만을 시행하고 추적 관찰한 군, 두 집단을 합한 군으로 나누어 임상적, 조직학적 인자들과의 관련성을 조사하였다.

면역조직화학염색법을 이용한 실험에서는 2001년부터 2012년까지의 41 개의 시편을 대상으로, 조직병리학적 조사를 시행하였다. 41 개 시편을 VEGF-c, c-Met, COX-2, Podoplanin, ROR1 의 항체를 이용하여 면역조직화학적 연구를 시행하였다.

생존율 분석을 위해서 Kaplan Meier method를 이용하였고, 잠재성 림프절 전이와 임상적, 병리학적, 면역조직화학적인 요소들과 관련성을 알아보기 위해 chi-square 와 Fisher's exact SPSS 통계 시스템 (version 21)이 사용되었다.

연구 결과

임상적인 소견

109명의 환자 중 남자 81명, 여자 28명의 분포를 보였으며, 연령은 23세에서 91세 범위에 있었고, 평균 연령은 54.4±15.4세였다. 40-60대의 환자에서 림프절 전이가 높게 나타났다. 종물제거술과 예방적 경부청소술을 동시에 받은 환자는 71명이었으며, 13명에서 림프절 전이가 관찰되었다. 종물 제거술만 받은 환자는 38명이었으며, 술 후 경부에 림프절 전이를 보인 환자는 8명이었다. 결국 예방적 경부청소술군, 추적관찰군, 통합군의 림프절 전이 발생률은 각각 18.3%, 21.1%, 19.3% 로 조사 되었다.

조직학적 소견

T2-3 군에서 림프절 전이가 높게 나타났으며, 예방적 경부청소술군에서 림프절 전이와의 통계적 유의성이 관찰 되었다. (P=0.017) 원발병소의 depth of invasion이 3mm 이상일 때, 모든 군에서 림프절 전이가 높게 나타났으며, 통합 군에서 림프절 전이와의 통계적 유의성이 관찰되었다. (P=0.022) 원발병소에 따른 림프절 전이 부위는, 예방적 경부청소술군에서 설 측면 부위에서 51명 중 8명, 구강저 부위에서 18중 4명, 설근부에서 2명 중 1명 잠재성 림프절 전이가 관찰되었다. 추적관찰군에서 설 측면 부위에서 35명 중7명, 구강저 부위에서 3중 1명, 설근부에서 0명 림프절 전이가 관찰되었다. Moderate/poor differentiation 군에서 림프절 전이가 높게 나타났지만, 통계학적 유의성은 관찰되지 않았다. 예방적 경부청소술군에서 잠재성 림프절 전이의 위치를 보면,

level I에서 4명, level II에서 2명, level III에서 4명, level IV에서 1명이 관찰되었으며, 림프절 전이가 2개 이상의 level에서 관찰된 환자는 2명 (Lv II, III) 이었다. 추적관찰군에서 림프절 전이의 level을 보면, level I에서 2명, level II에서 2명, level III에서 1명이 관찰되었으며, 림프절 전이가 2개 이상의 level에서 관찰된 환자는 3명 (Lv II, III 2명, Lv I, III, IV 1명) 이었다.

생존율 분석

추적관찰 군에서 3년, 5년 생존율은 각각 88.4%, 84.3% 였고, 예방적 경부청소술군에서 3년, 5년 생존율은 75.8%, 71.9% 였다. 추적관찰 군의 생존율이 예방적 경부청소술군보다 생존율이 높게 조사되었다. 또한 림프절 전이가 없는 군에서 림프절 전이가 있는 군보다 생존율이 높게 조사 되었다.

면역조직학적 소견

VEGF-c와 c-Met은 통합 군에서 잠재성 림프절 전이와 통계적으로 유의한 관련성이 있었다 (P=0.043, P=0.009). ROR1은 통합 군과 예방적경부청소술군에서 잠재성 림프절 전이와 유의한 관련성이 있었다 (P=0.003, 0.013). VEFG-c, c-Met, ROR1을 제외한 표시자들은 유의성 있는 연관성을 얻지 못했다.

결론

T 병기가 높거나 종물의 두께가 3mm 이상 이면 림프절 전이가 높게 나타난 다는 것을 발견하였다. VEGF-c, c-Met, ROR1은 잠재성 림프절 전이와 유의성 있는 연관성을 발견하였다. 림프절 전이가 있을 때 생존율이 떨어진다는 것도 발견하였다. 또한 상당한 비율의 림프절 전이 및 심지어는 skip metastasis가 일어날 수 있다는 것을 보여주었다. 임상적, 조직학적, 면역염색화학적 인자들을 고려한다면, 종물제거술과 예방적 경부청소술을 동시에 시행할지, 종물절제술만 시행 후 추적관찰을 할지 결정하는데 도움이 될 것으로 판단되며, 추적관찰 시 면밀한 추적관찰이 필요하다고 할 수 있겠다.

주요어: 잠재성 림프절전이(Occult metastasis), 경부 재발(Neck recurrence), 예방적 경부청소술(Elective neck dissection), 추적관찰(Watchful waiting), 면역조직화학염색법 (Immunohistochemistry)

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