

Original Article

Expression of VEGF, HGF, IL-6, IL-8, MMP-9, Telomerase in Peripheral Blood of Patients with Head and Neck Squamous Cell Carcinoma

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Objectives. This study investigated the telomerase expression in peripheral blood mononuclear cells (PBMCs) and the relationship between the serum level of several soluble factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, interleukin (IL)-6, IL-8, and matrix metalloproteinase-9 and the clinicopathological features of patients with head and neck squamous cell carcinoma (HNSCC).

Methods. Peripheral blood samples were collected from 50 HNSCC patients and 15 normal controls. The telomerase activity in the PBMCs was measured by Telomere Repeat Amplification Protocols. The serum levels of the soluble factors were analyzed by enzyme-linked immunosorbent assay.

Results. The expression of telomerase in the PBMCs of HNSCC patients was significantly correlated with the N and American Joint Committee on Cancer (AJCC) stages. The serum VEGF level was significantly higher in the patients with an advanced T stage, N stage and AJCC stage. Serum VEGF was significantly related with the expression of telomerase in the PBMCs. The telomerase expression and the VEGF expression were shown to be independent factors associated with poor survival.

Conclusion. The telomerase expression in the PBMCs and the serum VEGF level of HNSCC patients were significantly correlated with the N stage, the AJCC stage and the prognosis.

Key Words. *Telomerase, Peripheral blood mononuclear cells, Vascular endothelial growth factors, Prognosis, Prognostic markers*

INTRODUCTION

Despite the advances that have been made in surgery, chemotherapy and radiation therapy, there has been limited improvement in the survival of patients with head and neck cancer. The molecular studies that have focused on the pathogenesis of head and

neck squamous cell carcinoma (HNSCC) have developed new approaches to identify the higher risk patients who have progression or recurrence of disease and to help patients with multimodalities and targeted therapy. The serum levels of cytokines and angiogenesis factors may be applicable as tumor markers for the early detection of HNSCC, and as biomarkers to predict the outcome of patients with HNSCC (1-3).

In our previous report (4), we reported that the detection of a telomerase expression in the peripheral blood mononuclear cells (PBMCs) of HNSCC patients was a simple method and telomerase is a very useful molecular marker for assessing the progression and prognosis of HNSCC. In addition, they suggested two possible mechanisms for the telomerase expression in the PBMCs of the HNSCC patients. First, a number of soluble factors are secreted either by the tumor cells themselves or by the surround-

• Received July 23, 2009

Accepted after revision September 10, 2009

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• This work was supported for two years by Pusan National University Research Grant.

ing stroma during invasion or metastasis. PBMCs can be activated by these soluble factors and these cells have the high rate of expressing telomerase. Second, PBMCs can be activated by the antigenic stimulation of tumor cells in metastatic lymph nodes.

From the studies concerned with the growth, inflammatory and angiogenesis factors related to HNSCC, we selected several potential pathologic and prognostic markers such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), interleukin (IL)-6 and IL-8, and matrix metalloproteinase (MMP)-9. VEGF, IL-6, and IL-8 were found to be secreted by many established and freshly cultured HNSCC cell lines and these factors were detectable by immunohistochemistry in tumor specimens (1-3, 5, 6). HGF and MMP-9 were detectable at increased concentrations in the serum of many patients with HNSCC in other studies (6, 7).

We designed this study to examine the telomerase expression of PBMCs and the serum level of these soluble factors in patient with HNSCC and in normal controls. Our aims were to investigate: 1) the level of the telomerase expression and serum level of several soluble factors in patient with HNSCC and as compared to normal controls, 2) the relationship between the clinical characteristics of patient with HNSCC and the levels of biomarkers, 3) whether the telomerase expressions in PBMCs are correlated with one other, and 4) whether these biomarkers are associated with survival.

MATERIALS AND METHODS

Patients

The subjects were 50 patients with HNSCC that was confirmed at Pusan National University Hospital during the period from January to December 2004. All the subjects signed an IRB approved informed consent form prior to participation in this study. The patients who showed a poor general condition, those who were previously treated for cancer, and those who had autoimmune disease were excluded. Of the 50 patients, 41 (82%) were men and 9 (18%) were women. Their median age was 61.5 yr (range, 41 to 80 yr). The primary sites was the larynx in 25 cases, the hypopharynx in 9 cases, the oropharynx in 7 cases, the oral cavity in 7 cases and the maxillary sinus in 2 cases.

All the treatment was performed with surgery and/or radiotherapy and with or without chemotherapy, as recommended by a multidisciplinary tumor board. Fourteen patients underwent resection surgery and corresponding neck dissection, and 9 received adjuvant radiotherapy. Twelve patients were received radiotherapy only. Twenty-four patients underwent concurrent chemoradiotherapy with weekly cisplatin and radiotherapy. We used the American Joint Committee on Cancer (AJCC) version 6 TNM criteria for delineating the stage. The normal controls were 10 healthy men and 5 healthy women aged 25 to 65 with no history of chronic systemic disease or malignancies. After comple-

tion of treatment, the patients underwent routine surveillance every 1 to 3 months. The median follow-up period for the surviving patients was 25 months (range, 10 to 45 months).

Separation of the PBMCs and serum from the peripheral blood

Blood samples (12 mL) were collected in heparinized tubes at the time of the initial evaluation from the normal controls and the HNSCC patients before surgery, chemotherapy and/or radiotherapy. The serum samples were obtained by centrifugation at 2,000 rpm for 20 min and they were stored at -80°C . The PBMCs were separated by a Ficoll Hypaque density gradient and they were stored at -80°C until use.

Measurement of the serum level of soluble factors

The serum levels of soluble factors (VEGF, IL-6, IL-8, MMP-9, and HGF) were analyzed by enzyme-linked immunosorbent assay (ELISA) using Quantikine Immunoassay Kits. ELISAs were performed according to the methods recommended by the manufacturer. After the development of the colorimetric reaction, the optical density (OD) at 450 nm was quantified by an eight-channel spectrophotometer (Biotek Systems, Winooski, VT, USA), and the OD readings were converted to picograms per milliliter (pg/mL) on the basis of the standard curves obtained with recombinant cytokine in each assay. Each sample was tested in duplicate. The cytokine concentrations between the replicates varied by less than 10% and they are represented as mean values.

Telomeric Repeat Amplification Protocol (TRAP) assay in the PBMCs

The measurement and analysis of the telomerase activity of the PBMCs were conducted through the use of the TRAP^{EZE}™ Telomerase Detection Kit (Oncor Co., Gaithersburg, MD, USA), which is a modified the TRAP assay by Kim et al. (8).

The PBMCs were resuspended in 100 μL of lysis buffer (CHAPS) and then they were incubated for 30 min at 4°C . To prepare the extracted tissue, the stored tissues were thawed and then they were immediately put into ice to keep them at 4°C . The lysates were centrifuged at $12,000 \times g$ for 20 min at 4°C . The supernatants were transferred into fresh Eppendorf tubes, and the protein concentration was measured. The concentrated protein was then diluted with $1 \times$ CHAPS lysis buffer to make a 1 $\mu\text{g}/\mu\text{L}$ solution.

The TS primer (5'-AATCCGTCGAGCAGAGTT-3') to be used for the TRAP reaction was labeled with γ - ^{32}P -ATP (3,000 Ci/mmol, 10 mCi/mL) and T4 polynucleotide kinase. The total amount of the TRAP reaction solution was 25 μL : 2.5 μL of the $10 \times$ TRAP buffer (200 mM Tris-HCl, pH 8.3, 15 mM MgCl_2 , 630 mM KCl, 0.5% Tween 20, 10 mM EGTA, 0.1% BSA), 0.5 μL of the $50 \times$ dNTPs mix (25 mM each of dATP, dTTP, dGTP, and dCTP), 1 μL of the ^{32}P -TS primer, 0.5 μL of the TRAP primer mix (RP primer, K1 primer, TSK1 template), 0.2 μL of Taq polymerase (5 units/ μL ; Takara Co., Otsu, Japan), 18.3 μL of distilled water

and 2 µL of the specimen. The solution was put to reaction at 30°C for 30 min in a thermal cycler (Mastercycler 5330, Eppendorf Co., Hamburg, Germany) so that the telomerase that is supposed to be included in the specimen could react. Next, the reac-

tion was terminated by heating for 30 sec at 94°C. Amplification of the reaction product was repeated 30 times; one unit of amplification consisted of 30 sec at 94°C and 30 sec at 60°C.

Loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 50% glycerol, 50 mM EDTA, pH 8.0) was put into each reaction tube, and electrophoresis was conducted on 12.5% polyacrylamide gel and 0.25 × TBE buffer. The results were analyzed by using a phosphorimager (Molecular Dynamics Co., Sunnyvale, CA, USA). The samples that showed only bands of 36 bp were determined to be negative. The samples with accompanying TRAP products that had become longer by units of 6 bp (for examples, 50 bp and 56 bp, 62 bp and 68 bp, in addition to 36 bp) were determined to be positive. The human kidney 239 cell lines were used for the positive control, and distilled water was used for the negative control.

Table 1. The expression of tumor markers in the normal controls and HNSCC group

Tumor markers	Normal (n=15)	Cancer (n=50)	P-value
Telomerase (+)	26.7% (4/15)	84.0% (42/50)	<0.001
VEGF	19.42 ± 0.64	199.48 ± 82.59	<0.001
HGF	0.89 ± 3.47	125.04 ± 327.80	0.056
IL-6	0.21 ± 0.58	15.15 ± 70.47	0.153
IL-8	154.46 ± 195.02	88.79 ± 223.36	0.451
MMP-9	3.78 ± 2.86	12.61 ± 8.88	<0.001

Data are mean ± SD (pg/mL).

HNSCC: head and neck squamous cell carcinoma; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; IL: interleukin; MMP: matrix metalloproteinase.

Statistical analysis

Pearson’s chi-square test or the Wilcoxon signed rank test was used to evaluate the associations between the telomerase activi-

Table 2. The association of telomerase, VEGF, IL-6, IL-8, HGF and MMP-9 with the clinicopathologic parameters of the HNSCC patients

Parameters	No.	Telomerase activity		VEGF	IL-6	IL-8	HGF	MMP-9
		N (%)	P (%)					
Gender								
Male	41	6 (14.6)	35 (85.4)	204.45 ± 85.30	17.91 ± 77.71	104.17 ± 244.41	125.46 ± 356.09	12.68 ± 7.77
Female	9	2 (22.2)	7 (77.8)	177.16 ± 69.08	2.59 ± 2.70	18.76 ± 13.03	123.13 ± 155.43	12.32 ± 13.47
Age								
<60	18	4 (22.2)	14 (77.8)	222.82 ± 94.71	4.51 ± 11.62	19.93 ± 20.10	66.78 ± 112.08	13.25 ± 7.27
≥60	32	4 (12.5)	28 (87.5)	186.35 ± 73.27	21.14 ± 87.59	127.53 ± 272.64	157.81 ± 399.85	12.25 ± 9.77
Histologic grade								
WD-MD	40	5 (12.5)	35 (87.5)	203.29 ± 82.42	17.25 ± 78.49	107.51 ± 246.69	138.72 ± 364.51	18.62 ± 7.49
PD	10	3 (30.0)	7 (70.0)	184.19 ± 85.89	6.75 ± 15.43	13.92 ± 10.34	70.28 ± 71.44	17.83 ± 12.20
T stages								
T1-T2	37	7 (18.9)	30 (81.1)	176.61 ± 68.84 [†]	6.93 ± 23.64	87.49 ± 223.44	160.13 ± 375.50	13.64 ± 9.60
T3-T4	13	1 (7.7)	12 (92.3)	264.54 ± 86.26 [†]	38.55 ± 133.42	92.52 ± 232.21	25.17 ± 34.06	9.69 ± 5.79
N stages								
N (-)	26	8 (30.8)	18 (69.2)*	160.05 ± 73.44 [§]	3.82 ± 9.92	112.97 ± 262.27	145.82 ± 425.54	13.54 ± 10.58
N (+)	24	0 (0.0)	24 (100)*	242.18 ± 70.78 [§]	27.43 ± 100.85	62.60 ± 173.62	102.52 ± 176.29	11.6 ± 6.67
AJCC stage								
I-II	20	7 (35.0)	13 (65.0) [†]	129.95 ± 39.26	4.28 ± 11.22	93.48 ± 243.35	180.05 ± 481.97	14.77 ± 11.33
III-IV	30	1 (3.3)	26 (96.7) [†]	245.83 ± 70.42	22.41 ± 90.40	85.67 ± 213.24	88.36 ± 160.90	11.18 ± 6.62
Site								
Larynx	25	2 (8.0)	23 (92.0)	179.93 ± 69.59	6.83 ± 27.42	134.54 ± 295.57	151.23 ± 436.17	12.38 ± 7.44
Hyopharynx	9	2 (22.2)	7 (77.8)	238.14 ± 116.01	3.74 ± 2.48	27.66 ± 26.19	139.23 ± 232.43	13.21 ± 12.10
Oropharynx	7	1 (14.3)	6 (85.7)	242.25 ± 81.46	69.28 ± 182.22	27.13 ± 27.11	51.74 ± 74.56	14.32 ± 7.84
Oral cavity	7	2 (28.6)	5 (71.4)	166.19 ± 63.52	9.04 ± 18.25	85.61 ± 189.56	119.21 ± 158.47	9.63 ± 10.45
Maxilla	2	1 (50.0)	1 (50.0)	236.52 ± 1.29	2.47 ± 3.49	18.99 ± 2.98	10.65 ± 11.27	17.30 ± 3.13

All the values of VEGF, IL-6, IL-8, HGF and MMP-9 are expressed as means ± SD (pg/mL).

*P=0.001; [†]P=0.009; [‡]P=0.001; [§]P<0.001; ^{||}P<0.001.

VEGF: vascular endothelial growth factor; IL: interleukin; HGF: hepatocyte growth factor; MMP: matrix metalloproteinase; HNSCC: head and neck squamous cell carcinoma; N: negative; P: positive; WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated; AJCC: American Joint Committee on Cancer.

ty of the PBMCs or the serum levels of VEGF, HGF, IL-6, IL-8, and MMP-9 and the clinicopathologic parameters, including age, gender, the tumor site, the extent of tumor (the T and N stages and the overall stage) and the histopathologic grade (well, moderately, and poorly differentiated). This analysis was also used to investigate the correlation between the telomerase activity of PBMCs and the serum levels of VEGF, HGF, IL-6, IL-8, and MMP-9 in the HNSCC patients.

We performed univariate analysis using the Kaplan-Meier method and log-rank testing to examine the predictive value of the telomerase activity of the PBMCs and the serum levels of these soluble factors for the 3 yr overall survival. The Cox proportional hazards model was used for multivariate analysis. All the *P*-values presented were 2-sided, and the significance level was set at less than 0.05.

RESULTS

Telomerase activity and the serum levels of VEGF and MMP-9

Regarding the expression of telomerase in the PBMCs of the HNSCC patients, 42 out of 50 (84%) were positive, and 8 cases (16%) were negative. Of the 15 normal volunteers, 4 (26.7%) were positive. The difference between the normal controls and the HNSCC group for the telomerase expression of the PBMCs was significant ($P < 0.001$) (Table 1). The serum levels of VEGF and MMP-9 of the HNSCC group were significantly higher than those of the normal control group ($P < 0.001$), but there were no significant differences in the IL-6, IL-8 and HGF levels between the two groups (Table 1).

Telomerase activity and clinicopathologic parameters

The expression of telomerase in the PBMCs of the patients was significantly correlated with the N classification ($P = 0.001$) and the AJCC stage ($P = 0.009$). The patients with lymph node metastasis and an advanced AJCC stage (stage III or IV) showed a high-

Table 3. The relationship between the telomerase expression and the levels of the serum biologic markers in the peripheral blood of HNSCC patients

Biomarker	Telomerase (-) (n=8)	Telomerase (+) (n=42)	<i>P</i> -value
VEGF	120.77 ± 54.87	214.47 ± 78.74	0.002
HGF	54.32 ± 36.75	138.51 ± 356.41	0.214
IL-6	2.61 ± 2.38	17.54 ± 76.80	0.282
IL-8	78.60 ± 176.40	90.74 ± 232.99	0.869
MMP-9	13.42 ± 12.86	12.50 ± 8.12	0.783

Data are mean ± SD (pg/mL).

HNSCC: head and neck squamous cell carcinoma; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; IL: interleukin; MMP: matrix metalloproteinase.

er positive telomerase expression (Table 2).

Serum levels of VEGF and clinicopathologic parameters

There was no significant relationship between the serum levels of HGF, IL-6, IL-8, and MMP-9 and the clinicopathologic parameters. However, the serum levels of VEGF were significantly higher in the patients with an advanced T stage (T3 or T4) ($P = 0.001$), lymph node metastasis ($P < 0.001$) and an advanced AJCC stage (stage III or IV; $P < 0.001$) (Table 2).

Correlation between the telomerase activity and serum levels of VEGF

There were no significant relationship between the mean serum levels of HGF, IL-6, IL-8, and MMP-9 and the expression of the telomerase activity of the PBMCs in the HNSCC patients. However, the mean serum VEGF level was significantly related with the expression of telomerase activity in the PBMCs ($P = 0.002$) (Table 3).

Survival analysis

As shown in Table 4, univariate analysis reveals that the 3-yr survival rates were significantly associated with the N classification ($P = 0.023$), the AJCC stages ($P = 0.040$), the telomerase expression ($P = 0.016$) and the VEGF expression ($P = 0.028$). The Kaplan-Meier overall survival curves related to these prognostic factors are shown in Fig. 1. On the multivariate analysis, the N classification ($P = 0.025$), the AJCC stages ($P = 0.042$), the telomerase expression ($P = 0.047$) and the VEGF expression ($P =$

Table 4. The prognostic factors of HNSCC patients

Characteristics	3 yr survival rate (%)	Univariate analysis (<i>P</i> -value)	Multivariate analysis (<i>P</i> -value)
Age (<60/>60)	83.3/75.0	0.271	0.275
Gender (male/female)	80.5/66.7	0.191	0.194
T classification (T1-2/T3-4)	86.5/53.8	0.072	0.074
N classification (N0/N+)	92.3/62.5	0.023	0.025
AJCC stage (I-II/III-IV)	95.0/66.7	0.040	0.042
Histologic grade (WD-MD/PD)	77.5/80.0	0.984	0.990
Telomerase (-/+)	100/73.8	0.045	0.047
VEGF (low/high)	92.3/62.5	0.028	0.029
HGF (low/high)	84.0/72.0	0.172	0.174
IL-6 (low/high)	76.9/79.2	0.870	0.874
IL-8 (low/high)	74.1/82.6	0.675	0.678
MMP-9 (low/high)	81.5/73.9	0.542	0.538

Low VEGF, HGF, IL-6, IL-8, and MMP-9 are the patients with lower levels than each of the mean levels, and high means higher levels than each of the mean levels.

HNSCC: head and neck squamous cell carcinoma; WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; IL: interleukin; MMP: matrix metalloproteinase.

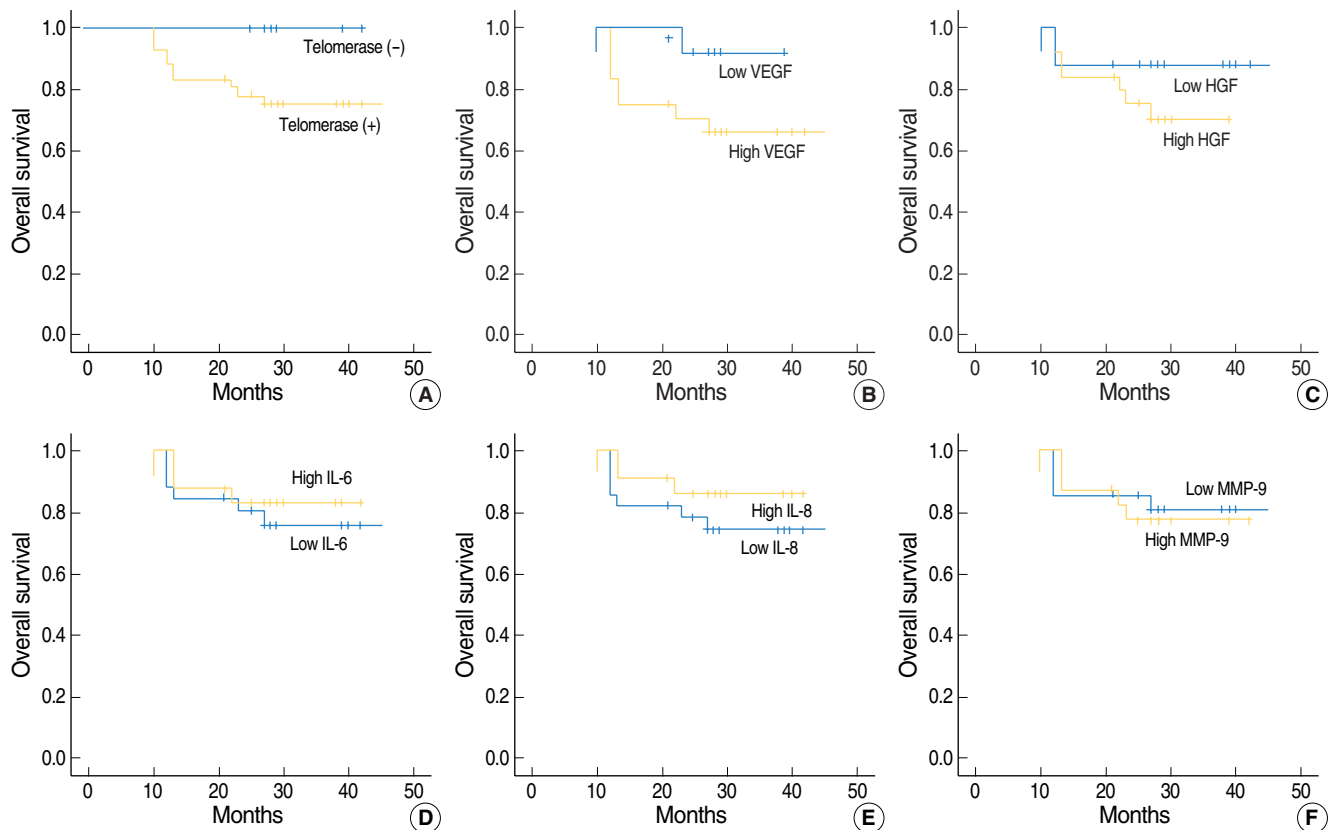


Fig. 1. Kaplan-Meier overall survival curves. There are significant differences in the Kaplan-Meier overall survival curves related to the telomerase expression and the serum vascular endothelial growth factor (VEGF) level. (A) Telomerase expression ($P=0.045$). (B) Serum VEGF level ($P=0.028$). (C) Serum hepatocyte growth factor level ($P=0.172$). (D) Serum interleukin (IL)-6 level ($P=0.870$). (E) Serum IL-8 level ($P=0.675$). (F) Serum matrix metalloproteinase-9 level ($P=0.542$).

0.029) were shown to be independent factors associated with poor survival (Table 4).

DISCUSSION

The expression of telomerase activity in HNSCC tissue is a useful indicator to determine the progression of disease and the prognosis of these patients. Although telomerase activity has been detected in normal human PBMCs, increased telomerase activity has been observed in the PBMCs of HNSCC patients (4, 9). In this study, the rate of the telomerase expression in the PBMCs of the HNSCC patients was significantly higher than that of the healthy volunteer control group ($P<0.001$). The telomerase expression in the PBMCs of the patients was significantly correlated with the N classification and the AJCC stage ($P<0.05$). These findings are consistent with the results of Lee et al. (4). There have been many reports on the activity of telomerase in the various types of cancer patients (10-14). Compared with the existing method for extracting tissues specimen, this method that evaluates the telomerase activity in PBMCs is less invasive, safer and more convenient because it needs only blood samples of patients.

There have been some studies that cytokines and angiogenesis factors may be potential useful as serum markers for the prognosis, progression, recurrence and survival of patient with HNSCC (1-3, 5). We examined the serum levels of VEGF, MMP-9, HGF, IL-6, and IL-8, which are well-known for their roles in the development, progression and invasive growth of tumors (5). Although the VEGF ($P<0.001$) and MMP-9 ($P<0.001$) levels were significantly higher than those of the normal control group, no significant differences of the others factors were observed between the HNSCC group and the normal control group. However, Druzgal et al. (1) reported that the serum levels of IL-6, IL-8, HGF, VEGF and growth regulated oncogene were increased in the patients with HNSCC as compared with those of the normal control group. Gokhale et al. (6) reported that the serum level of IL-8 of the HNSCC group was significantly different from that of the normal control group, but the serum level of VEGF showed no significant difference between the normal control group and the HNSCC group. Hathaway et al. (2) reported that the serum levels of IL-1b, tumor necrosis factor- α and IL-6 were higher in HNSCC patients than those of chronic smokers. Ruokolainen et al. (7) reported that the MMP-9 serum levels were significantly higher in the HNSCC patients than those in the healthy controls. We suggest that the

serum VEGF and MMP-9 levels may be helpful for making an early diagnosis and for detecting recurrence. Further study is needed for the patients who have finished treatment and/or are suspected to have recurrence.

Although the serum level of MMP-9 was significantly higher than that of the normal control group, there was no correlation between the serum level of other cytokines (IL-6, IL-8, and HGF) and the clinicopathologic features of the HNSCC group. The expression of VEGF was higher for the late T stage patients than that for the early T stage patients and it was increased in the patients with lymph node metastasis and an advanced AJCC stage. This means that the expression of serum VEGF is increased in patient with an advanced HNSCC stage. On the univariate and multivariate analyses ($P=0.028$, $P=0.029$), the VEGF level was an independent factor associated with poor survival. Kumar et al. (15) presented that the significant increase of the serum VEGF level is related to the clinical stage and lymph node metastasis in patients with colorectal cancer. Teknos et al. (16) reported that patients with advanced laryngeal carcinoma showed significantly higher serum VEGF levels than did the healthy controls, and elevated serum VEGF levels tended to indicate a more aggressive disease state and poorer overall survival. Ruokolainen et al. (7) reported that no correlation was found between the MMP-9 serum levels and the histopathologic factors. Hathaway et al. (2) reported that the serum levels of IL-8 significantly differed by the T stage of HNSCC patients, IL-4 differed by the N stage and IL-6 showed no significant difference by the T and N stages. Druzgal et al. (1) reported that the serum levels of IL-6, IL-8, HGF, and VEGF were increased in patients with HNSCC as compared to the normal control group, but there was no significant relationship between the serum levels of these factors and the disease-free survival or overall survival. Further studies should be considered to obtain more significant results.

This study showed a significant relationship between the expression of telomerase in PBMCs and the serum VEGF that may be secreted by the tumor cells. This result supports the first suggestion of Lee et al. (4) that the telomerase expression in PBMCs could be activated by several soluble factors, including VEGF. Chiu and Harley (17) maintained that normal blood cells and other somatic cells, including mature and stable lymphocytes, rarely show a telomerase expression, but the activated mononuclear cells in the peripheral blood such as T and B lymphocytes can show a telomerase expression by antigenic stimulation. In an experimental animal model of hind limb ischemia, Zaccagnini et al. (18) reported that VEGF causes the activation of telomerase through the nitric oxide pathway in the process of new vessel formation. Especially, Shao and Guo (19) mentioned that human telomerase reverse transcriptase, which is a telomerase catalytic subunit, behaves as an angiogenic factor and a downstream effector of VEGF signaling.

The growth factors produced by the tumor cells act in an autocrine manner by stimulating tumor cell migration, invasion and

proliferation. They also induce chemotaxis, proliferation and differentiation of inflammatory cells. Several cytokines and growth factors that are secreted by the cells of the immune system often act in concert to mediate immune and inflammatory responses, and this leads to recruitment and activation of lymphocytes and inflammatory cells at the sites of tumor. Although there have been many studies about the roles and functions of these soluble cytokines and growth factors at disease sites, there have been few reports about the role of circulating soluble cytokines and the relationship between PBMCs and these factors in solid tumors. The results of this current study suggest the possibility that the serum levels of VEGF might be applicable as a reflection of the disease status in HNSCC patients. Further, this study showed a significant relationship between the expression of telomerase in PBMCs and the serum VEGF. Further studies will be required to ascertain the mechanism by which the telomerase activation of PBMCs is activated by circulating soluble growth factors.

The telomerase expression in the PBMCs of HNSCC patients was significantly correlated with the N stage, the AJCC stage and the prognosis. Although both the serum VEGF and MMP-9 levels of the HNSCC group were significantly higher than those of the normal control group, only the serum VEGF level showed a significant difference according to the T stage, the N stage, the AJCC stage and the prognosis. This study showed a significant relationship between the expression of telomerase in PBMCs and the serum VEGF level. Further studies are required to assess the relationship and interaction between serum VEGF and the telomerase expression in PBMCs.

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