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Original Article

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Is Human Kallikrein 11 in Non-small Cell Lung Cancer Treated Chemoradiotherapy Associated with Survival?

Dilek Unal, MD[1]
Celalettin Eroglu, MD[2]
Arzu Tasdemir, MD[3]
Hatice Karaman, MD[3]
Neslihan Kurtul, MD[4]
Arzu Oguz, MD[5]
Sema Sezgin Goksu, MD[5]
Bunyamin Kaplan, MD[2]

[1]*Department of Radiation Oncology, Kayseri Education and Research Hospital, Kayseri,* [2]*Department of Radiation Oncology, Erciyes University Medical Faculty, Kayseri,* [3]*Department of Pathology, Kayseri Education and Research Hospital, Kayseri,* [4]*Department of Radiation Oncology, Sutcu Imam University Medical Faculty, Kahramanmaras,* [5]*Department of Medical Oncology, Kayseri Education and Research Hospital, Kayseri, Turkey*

Correspondence: Dilek Unal, MD
Department of Radiation Oncology, Kayseri Education and Research Hospital, Kayseri Eğitim ve Araştırma Hastanesi, Radyasyon Onkolojisi Kliniği, 38010, Kayseri, Turkey
Tel: 90-352-3368884
Fax: 90-352-4375807
E-mail: dilekunaldr@gmail.com

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Purpose

Involvement of human kallikreins (hKs) in human cancers has been reported and several hKs are promising biomarkers of various cancers. The aim of this study was to evaluate the prognostic significance of hK11 expression in patients with non-metastatic non-small cell lung cancer (NSCLC).

Materials and Methods

The study included 44 patients with NSCLC. hK11 expression was determined by immunohistochemical staining.

Results

The estimation of disease-free and overall survival by Kaplan-Meier was 11 months and 17 months, respectively. The estimation of overall survival by Kaplan-Meier was significantly higher in patients with hK11 strongly positive (2+) than in those with hK11 weakly positive (1+) (20 months vs. 11 months, p=0.032). Although not statistically different, the estimation of disease-free survival by Kaplan-Meier was higher in patients with hK11 strongly positive (2+) than in those with hK11 weakly positive (1+) (12 months vs. 9 months, p=0.113). Multivariate Cox regression analysis showed that the overall survival rates were significantly associated with response to chemoradiotherapy and the degree of staining with hK11.

Conclusion

The stronger hK11 expression in NSCLC appears to be associated with better survival rates. hK11 may be a prognostic biomarker of NSCLC.

Key words
Kallikrein-11, Lung neoplasms, Survival

Introduction

Lung cancer is the leading cause of cancer-related deaths. According to the World Health Organization (WHO), it is divided into two categories based on its biology, therapy and prognosis as small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC, accounting for more than 85% of all lung cancer cases, consists of two major histological subtypes; squamous and non-squamous (adeno, large cell and other cell types). Only about 15.6% of all lung cancer patients are alive 5 years or more after diagnosis [1,2]. The majority of patients with lung cancer are at the advanced stage at time of diagnosis. Therefore, late diagnosis is still a

problem in the effort to improve the outcomes and early stage at diagnosis is an important prognostic factor [3]. Defining the high risk group patients is important for early diagnosis and chance of cure [2].

New biological markers, which can predict patients with higher risk and poor outcome, can be a new therapeutic approach. The potential uses of the markers include aiding early diagnosis, determining prognosis, prospectively predicting response or resistance to specific therapies, and monitoring therapy in patients with advanced disease [4,5]. Kallikreins, a subgroup of the serine protease enzyme family, are expressed in many tissues, including steroid hormone-producing or hormone-dependent tissues such as the prostate, breast, ovary, and testis. Human tissue kallikreins are a family of 15 highly conserved serine proteases encoded by the largest contiguous cluster of protease genes in the human genome [6,7]. Measurement of kallikrein genes and proteins in serum and tumor tissue extracts may be useful for the purpose of disease diagnosis, monitoring, prognosis, or subclassification [4,6]. Borgono et al. [8] showed that human kallikrein 11 (hK11) positivity is associated with a slower disease progression and hK11 is an independent marker of favorable prognosis in patients with ovarian cancer. In a recent study, we observed that hK11 expression in gastric cancer is associated with a better prognosis [4]. In the current study, we aimed to evaluate the prognostic value of immunohistochemical expression of hK11 in patients with non-metastatic NSCLC treated with chemoradiotherapy.

Materials and Methods

1. Patient population

A total of 88 eligible patients with non-metastatic NSCLC were treated with chemoradiotherapy. Patients who were < 18 years old, had severe disease such as heart failure, renal failure, or hepatic failure, a history of any other cancer, or a metastatic disease were excluded. The diagnosis of lung cancer was made by bronchoscopic biopsy in most cases. Therefore, the majority of patients did not have adequate biopsy specimens for immunohistochemical evaluation. Finally, 44 patients were included in the study. Forty-one of the patients (93.2%) were male and only three patients (6.8%) were female. The mean age was 57±6 years.

We recorded responses to chemoradiotherapy including complete remission, regression, stable disease, and disease progression, and overall and disease-free survival. Survival time was measured from the date of chemoradiotherapy until death or last clinical evaluation.

Histopathology of NSCLC was squamous cell carcinoma in 30 (68.2%), adenocarcinoma in eight (18.2%), large cell carcinoma in three (6.8%), and unclassified NSCLC in three (6.8%) patients.

2. Radiotherapy and chemotherapy

Two-dimensional treatment planning system was used by conventional X-ray simulator and radiotherapy was delivered by a linear accelerator device including 6-18 million volts photons. Total dose of 66 Gy in 2.0 Gy fractions was given. During radiotherapy weekly docetaxel 20 mg/m^2 and cisplatin 20 mg/m^2 infusion were administered concomitantly on the first day of every week for 6.5 weeks. The initial planning target volume consisted of the primary tumor, the ipsilateral hilum, and mediastinum with a margin of 2 cm. Special blocks were employed in order to prevent exposure of normal tissues to radiation. This initial field was treated by parallel-opposed anterior and posterior fields to 46 Gy in 23 fractions. Boost dose of radiotherapy was administered after 46 Gy to the primary tumors and the involved nodes were included with a margin of 0.5-1.5 cm from oblique parallel-opposed fields with protecting the spinal cord.

After chemoradiotherapy, complete remission developed in 11 patients (25.0%), regression in 16 (36.4%), stable disease in seven (15.9%), and disease progression in 10 (22.7%). Stable disease or disease progression after chemoradiotherapy was defined as lack of response to chemoradiotherapy. In contrast, complete response or disease regression after chemoradiotherapy was defined as response to chemoradiotherapy. A response to chemoradiotherapy was observed in 27 patients (61.4%) whereas 17 patients (38.6) did not respond to chemoradiotherapy.

3. Immunohistochemistry

Tissues were fixed in 10% buffered formalin and embedded in paraffin. One paraffin-embedded block tissue was selected from each case and cut into 4-μm sections. Tissue sections were deparaffinized with xylene and washed with ethanol. Commercially available rabbit antihuman polyclonal antibody of hK11 (lot No. 82067, Biorbyt, Cambridge, UK) was used. Immunohistochemical staining was performed using avidin-biotin-peroxidase method.

Positive and negative immunohistochemical controls were routinely used. hK11 positivity was defined as the cytoplasm and cytoplasmic membrane staining in at least 10% of tumor cells. hK11 negativity was defined as cytoplasmic staining in less than 10% of tumor cells. The staining density equal to or less than control specimens was defined as weakly positive (1+) (Fig. 1A). In contrast, more intense staining of control specimens was defined as strongly positive (2+) (Fig. 1B).

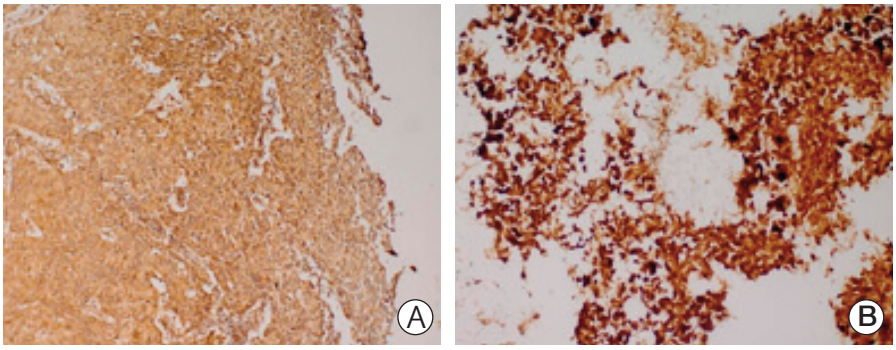

Fig. 1. Positive staining with human kallikrein 11 in cytoplasm and cytoplasmic membrane in lung cancer cells: (A) weakly positive (1+), (B) strongly positive (2+).

Table 1. Clinical and pathological characteristics of the patients with human kallikrein 11 weakly positive (1+) and strongly positive (2+)

Characteristic	Total (n=44)	Weakly positive (1+) (n=17)	Strongly positive (2+) (n=27)	p-value
Age (yr)	57±6	57.1±6.7	57.6±6.1	0.809
Sex				
Female	3 (6.8)	1 (5.9)	2 (7.4)	0.671
Male	41 (93.2)	16 (94.1)	25 (92.6)	
Histology				
Squamous	30 (68.2)	13 (76.5)	17 (63.0)	0.275
Non-squamous	14 (31.8)	4 (23.5)	10 (37.0)	
T status				
T1-2	11 (25.0)	4 (23.5)	7 (25.9)	0.576
T3-4	33 (75.0)	13 (76.5)	20 (74.1)	
N status				
N0	19 (43.2)	7 (41.2)	12 (44.4)	0.541
N1-3	25 (56.8)	10 (58.8)	15 (55.6)	
Stage				
II	4 (9.1)	-	4 (14.8)	0.229
IIIA	18 (40.9)	7 (41.2)	11 (40.7)	
IIIB	22 (50.0)	10 (58.8)	12 (44.4)	
Response to chemoradiotherapy[a)]				
Presence	27 (61.4)	10 (58.8)	17 (63.0)	0.515
Absence	17 (38.6)	7 (42.2)	10 (37.0)	

Values are presented as number (%). [a)]Presence: complete response or disease regression/absence: stable disease or progression.

Immunohistochemical staining with hK11 was positive in all patients. Seventeen patients (38.6%) had weakly positive (1+) with hK11 whereas 27 patients (61.4%) had strongly positive (2+) with hK11.

4. Statistical analysis

SPSS ver. 15.0 (SPSS Inc., Chicago, IL) was used for the statistical analysis. Continuous variables with normal distribution were presented as mean±standard deviation. Statistical analysis for the parametric variables was performed

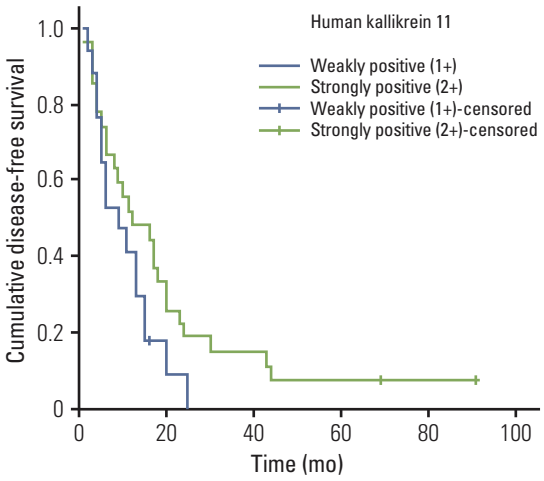

Fig. 2. The disease-free survival according to the degree of staining with human kallikrein 11.

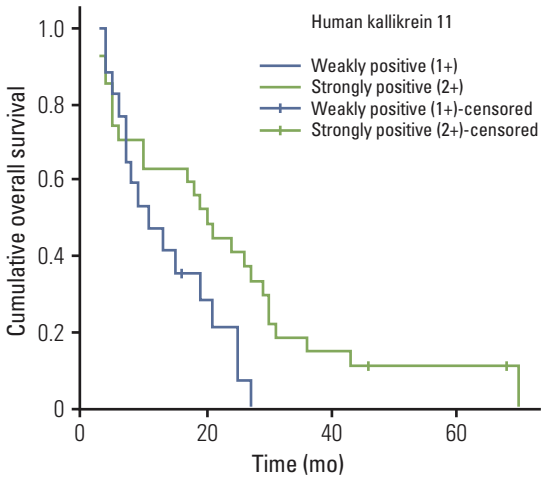

Fig. 3. The overall survival according to the degree of staining with human kallikrein 11.

using the Student's t test between two groups. Qualitative variables are given as percent and the correlation between categorical variables was determined using the chi-square test and Fisher exact tests. Disease-free survival and overall survival were estimated using the Kaplan-Meier method and the log-rank test was used for comparison of outcomes. Mortality risks were analyzed using the multivariate Cox regression model in which we included (in a backward-wald manner) all significant variables from the univariate analysis. A p-value of < 0.05 was considered significant.

Results

Table 1 shows clinical and pathological characteristics of the patients with hK11 weakly positive (1+) and strongly positive (2+). There was no significant difference between two groups in terms of age, sex, cancer histology, T and N status, stage, and response to chemoradiotherapy ($p > 0.05$).

The estimation of disease-free and overall survival by Kaplan-Meier was 11 months and 17 months, respectively (data not shown).

Fig. 2 shows disease-free survival according to the degree of staining with hK11. Although not statistically different, the estimation of disease-free survival by Kaplan-Meier was higher in patients with hK11 strongly positive (2+) than in those with hK11 weakly positive (1+) (12 months vs. 9 months, p=0.113).

Fig. 3 shows overall survival according to the degree of staining with hK11. The estimation of overall survival by Kaplan-Meier was significantly higher in patients with hK11 strongly positive (2+) than in those with hK11 weakly positive (1+) (20 months vs. 11 months, p=0.032).

The overall survival rates according to characteristics of the patients are shown in Table 2. Histology of cancer, T status, response to chemoradiotherapy, and the degree of staining with hK11 were the characteristics that significantly influenced the overall survival (p=0.021, p=0.038, p=0.011, and p=0.032, respectively).

The overall survival and p-values adjusted for patient characteristics in patients with hK11 weakly positive (1+) and strongly positive (2+) are shown in Table 3. A significant difference in the overall survival adjusted for age groups, sex, T status, and N status was observed between the two groups (p=0.043, p=0.026, p=0.041, and p=0.039, respectively). However, no significant difference in the overall survival adjusted for histology and response to chemoradiotherapy was observed between the two groups ($p > 0.05$).

Univariate and multivariate analyses were performed to determine the risk factor(s) related to overall survival. Table 4 shows the results regarding eight variables examined in univariate analysis as potential risk factors for overall survival. In univariate analysis four of the eight factors (histology of cancer, T status, response to chemoradiotherapy, and the degree of staining with hK11) differed significantly between these groups ($p < 0.05$). All of these significant variables in the univariate analysis were included in the multivariate Cox regression for analysis of mortality risk. Multivariate Cox regression analysis determined that the overall survival rates were significantly associated with

Table 2. Overall survival and p-value according to characteristics of patients

Characteristic	No. of patients (%)	Survival (95% CI, mo)	p-value
Age (yr)			
≥ 65	6 (13.6)	17 (0-36.2)	0.849
< 65	38 (86.4)	15 (5.65-24.35)	
Sex			
Female	3 (6.8)	10 (8.40-11.60)	0.686
Male	41 (93.2)	18 (10.72-25.28)	
Histology			
Squamous	30 (68.2)	10 (0.61-19.39)	0.021
Non-squamous	14 (31.8)	25 (12.17-37.83)	
T status			
T1-2	11 (25.0)	26 (22.76-29.24)	0.038
T3-4	33 (75.0)	13 (5.59-20.42)	
N status			
N0	19 (43.2)	11 (3.89-18.11)	0.332
N1-3	25 (56.8)	21 (13.23-28.77)	
Stage			
II	4 (9.1)	19 (0.07-37.94)	0.892
IIIA	18 (40.9)	17 (10.11-23.90)	
IIIB	22 (50.0)	5 (0-10.88)	
Response to chemoradiotherapy[a)]			
Presence	27 (61.4)	21 (17.36-24.64)	0.011
Absence	17 (38.6)	6 (3.99-8.02)	
Human kallikrein 11			
Weakly positive (1+)	17 (38.6)	11 (4.28-17.72)	0.032
Strongly positive (2+)	27 (61.4)	20 (14.92-25.09)	

CI, confidence interval. [a)]Presence: complete response or disease regression/absence: stable disease or progression.

response to chemoradiotherapy and the degree of staining with hK11. In contrast, the overall survival rates did not show significant association with histology of cancer and T status ($p > 0.05$).

Discussion

Interactions occur between serine proteases and substrates of serine proteases and many other substrates, including extracellular matrix proteins. Serine proteases play a role in cascade pathways, such as the blood coagulation cascade, the dissolution of blood clots, the intestinal digestive enzymes, and the apoptosis pathway [6]. Kallikrein cascade seems to be valuable in elucidation of the various mechanisms underlying cancer invasion and metastasis [9]. There is evidence of a link between kallikreins and cancer. Prostate-specific antigen (PSA; hK3) is the most used tumor marker [6]. The most successful clinical use of PSA is currently in the diagnosis and monitoring of prostate cancer [10]. Similarly, kallikreins such as hK4, hK6, hK9, hK10, hK11, and hK12 are emerging new markers for the diagnosis and prognosis of various tumors.

Previous studies have reported an association between kallikreins and cancer prognosis. Yu et al. [9] reported that over-expression of hK11 was associated with poor prognosis in patients with low rectal carcinoma. Diamandis et al. [11] found that hk11 is an indicator of favorable prognosis in ovarian cancer patients. They observed that patients with hK11-positive tumors had a significantly longer progression-free survival and overall survival and hK11 was an independent prognostic indicator of progression-free survival [11]. Similarly, in another study conducted by these authors in patients with ovarian cancer, it was found that hK11-positive tumors were more frequently associated with early stage disease, pre-/peri-menopausal status and patients who exhibited complete or partial response to chemotherapy and patients with hK11-positive tumors had a significantly decreased risk of relapse and death [8].

In the literature, there are a limited number of studies on

Table 3. Overall survival and p-value in patients with human kallikrein 11 weakly positive (1+) and strongly positive (2+) according to characteristics of patients

Characteristic	95% CI (mo)		p-value
	Weakly positive (1+)	Strongly positive (2+)	
Age (yr)			
≥ 65	5	21 (12.41-29.59)	0.043
< 65	11 (3.16-18.84)	19 (2.91-35.09)	
Sex			
Female	9	10	0.026
Male	11 (1.20-20.80)	20 (15.10-24.90)	
Histology			
Squamous (%)	13 (4.78-21.22)	10 (0-27.48)	0.113
Non-squamous (%)	7 (2.10-11.90)	29 (24.35-33.65)	
T status			
T1-2 (%)	6 (0-25.60)	30 (19.74-40.27)	0.041
T3-4 (%)	11 (5.13-16.87)	17 (0-34.53)	
N status			
N0 (%)	11 (3.30-18.70)	10 (0-32.07)	0.039
N1-3 (%)	9 (0-28.28)	24 (15.16-32.84)	
Stage			
II (%)	-	24 (6.74-41.26)	β
IIIA (%)	11 (0.74-21.26)	20 (14.90-25.09)	
IIIB (%)	9 (1.25-16.75)	5 (0-10.88)	
Response to chemoradiotherapy[a)]			
Presence (%)	15 (3.98-26.02)	21 (14.28-27.72)	0.067
Absence (%)	7 (4.65-9.34)	5 (2.93-7.07)	

CI, confidence interval; β, no pooled comparison is performed because at least one stratum does not have valid cases for each factor level. [a)]Presence: complete response or disease regression/absence: stable disease or progression.

Table 4. Univariate and multivariate analysis of risk factors for overall survival

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (< 65 yr or ≥ 65 yr)	1.09 (0.46-2.60)	0.852	-	-
Sex (female or male)	1.27 (0.39-4.17)	0.693	-	-
Histology (squamous or non-squamous)	2.19 (1.09-4.40)	0.028	-	-
T status (T3-4 or T1-2)	2.17 (1.01-4.64)	0.047	-	-
N status (N0 or N1-3)	1.36 (0.72-2.54)	0.345	-	-
Stage (II or IIIA or IIIB)	-	0.897	-	-
Response to chemoradiotherapy (absence or presence)[a)]	2.30 (1.17-4.25)	0.015	2.35 (1.22-4.51)	0.010
Human kallikrein 11 (weakly positive [1+] or strongly positive [2+])	2.08 (1.04-4.18)	0.039	2.23 (1.10-4.56)	0.027

OR, odds ratio; CI, confidence interval. [a)]Absence: stable disease or progression/presence: complete response or disease regression.

the importance of hK11 in lung cancer. Planque et al. [7] examined the clinical value of 11 members of the hK family as potential biomarkers for the diagnosis of lung cancer. They obtained serum specimens from 51 patients with NSCLC and from 50 healthy subjects. They found that patients with NSCLC had lower levels of KLK5, KLK7,

KLK8, KLK10, and KLK12, and higher levels of KLK11, KLK13, and KLK14 compared to controls and proposed a multiparametric panel of kallikrein markers for the diagnosis of NSCLC with relatively good accuracy [7]. Similarly, Xu et al. [5] evaluated the diagnostic and prognostic value of serum human kallikrein–related peptidases 11 level in NSCLC. They collected serum specimens from 138 patients with NSCLC and 40 healthy controls. They observed that serum human kallikrein–related peptidases 11 levels were significantly higher in NSCLC compared to that in the controls and NSCLC patients with serum high human kallikrein–related peptidases 11 had a longer overall survival and progression-free survival than those with low human kallikrein–related peptidases 11 and concluded that serum human kallikrein–related peptidases 11 might be a useful diagnostic and prognostic test for NSCLC patients [5].

In the current study, we observed that the stronger hK11 expression was associated with better survival rates in patients with NSCLC. Overall survival rates were significantly higher in patients with hK11 strongly positive (2+) compared to those with hK11 weakly positive (1+). In addition, although not statistically different, disease-free survival rates were higher in patients with hK11 strongly positive (2+) compared to those with hK11 weakly positive (1+).

Cancer cells and some cells of the immune system produce substances that induce apoptosis in normal tissues. Apoptosis is genetically regulated and may be corrupted in malignant cells. For example, the p53 tumor suppressor gene stimulates apoptosis, whereas the Bcl-2 oncogene inhibits it [12]. Apoptosis pathway is an important example of coordinated action of serine protease [6]. hK11 may improve the prognosis by enhancing chemoradiotherapy-induced apoptosis in lung cancer.

In our recent study on the value of hK11 expression in gastric cancer, we found that negativity in tumor negatively affects the prognosis [4]. Similarly, in the current study, we found that prognosis worsens as staining with hK11 in lung cancer tissue is decreasing. On the other hand, we found no negative staining with hK11 in lung cancer tissue.

Conclusion

hK11 may be a prognostic biomarker of NSCLC, which is usually diagnosed at advanced stage, and new therapeutic and prognostic indicators are needed. The stronger hK11 expression in NSCLC appears to be associated with better survival rates. On the other hand, the mechanisms underlying the regulation of hK11 expression in NSCLC should be elucidated.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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