The Role of Serum Adhesion Molecules and Vascular Endothelial Growth Factor in Lung Cancer Patients

Akciğer Kanserli Hastalarda Serum Adezyon Molekülleri ile Vasküler Epitelyal Büyüme Faktörünün Rolü

Ahmet Selim YURDAKUL, MD,[a]
Elif Reyhan HAN, MD,[a]
Neslihan BUKAN, MD,[b]
Can ÖZTÜRK, MD[a]

Departments of
[a]Pulmonary Medicine,
[b]Biochemistry,
Gazi University Faculty of Medicine,
Ankara

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Yazışma Adresi/*Correspondence:*
Ahmet Selim YURDAKUL, MD
Gazi University Faculty of Medicine,
Pulmonary Medicine, Ankara,
TÜRKİYE/TURKEY
ayurdakul@gazi.edu.tr

ABSTRACT Objective: Matrix metalloproteinase (MMP) and vascular endothelial growth factor (VEGF) which are involved in anjiogenesis and serum intercellular adhesion molecule (sICAM) which is a transmembranous protein from the immunoglobulin family play important role in tumor progression. The aim of this study was to analyze the association of serum levels of MMP-9, MMP-13, VEGF and sICAM with clinical parameters and survival in patients with non-small-cell lung cancer (NSCLC), and to determine whether these proteins might be useful tumor markers for lung cancer. **Material and Methods:** In our study, serum levels of MMP-9, MMP-13, VEGF and sICAM were analysed in 72 patients with NSCLC patients (mean age: 60.03±10.86) and 46 healthy controls (mean age: 61.13 ± 14.71). The analysis was performed by enyzme-linked immunosorbent assay (ELISA). **Results:** The median survival of all patients was 22 months. Serum levels of sICAM, VEGF and MMP-9 were increased in NSCLC patients (985 ± 489.4, 248.7±255.9, 5148.2±1996.2) compared with the healthy controls (300.5 ± 204.1, 182.1 ± 207.5, 2943.6 ± 851.7), whereas MMP-13 levels were not significantly different from healthy control group (0.46 ± 0.48, 0.53 ± 1.35) ($p > 0.05$). No statistically significant relationships were found between all investigated serum parameters and age, smoking, Eastern Cooperative Oncology Group performance status, or gender ($p > 0.05$). Additionally, there was no significant association between all serum parameters levels and survival in NSCLC patients ($p > 0.05$). **Conclusion:** High levels of MMP-9, VEGF and sICAM were found in NSCLC patients, however there was no statistically significance in MMP-13 levels. Analysis of serum levels of MMP-9, VEGF and sICAM appears as a potential tumor marker.

Key Words: Vascular endothelial growth factors; lung; matrix metalloproteinases; intercellular adhesion molecule-1

ÖZET Amaç: Anjiogenezis içinde yer alan matriks metalloproteinaz (MMP) ile vasküler endotelyal büyüme faktörü (VEGF) ve immunglogulin ailesi içinde yer alan transmembranöz bir protein olan dolaşımdaki intersellüler adezyon molekülleri (sICAM) tümör progresyonunda önemli rol oynar. Bu çalışmanın amacı, küçük hücreli dışı akciğer kanserli (KHDAK) hastalarda tedavi öncesi ölçülen MMP-9, MMP-13, VEGF ve sICAM serum düzeyleri ile hastaların klinik parametreleri ve yaşam süreleri arasındaki ilişkiyi analiz etmektir. **Gereç ve Yöntemler:** Çalışmamızda 72 KHDAK'li hasta (ort. yaş: 60.03 ± 10.86) ile 46 sağlıklı kontrol grubunda (ort. yaş: 61.13 ± 14.71) MMP-9, MMP-13, VEGF ve sICAM serum düzeyleri analiz edildi. Analizler ELİSA yöntemi ile yapıldı. **Bulgular:** KHDAK'li hastaların medyan yaşam süresi 22 ay olarak bulundu. MMP-9, VEGF ve sICAM serum seviyeleri sağlıklı kontrol grubu (300.5 ± 204.1, 182.1 ± 207.5, 2943.6 ± 851.7) ile karşılaştırıldığında, KHDAK'li hastalarda daha yüksek olarak bulundu (985.0 ± 489.4, 248.7 ± 255.9, 5148.2 ± 1996.2). Ancak MMP-13 düzeylerinde sağlıklı kontrol grubuna göre anlamlı bir farklılık saptanmadı (0.46 ± 0.48, 0.53 ± 1.35)($p > 0.05$). Araştırılan tüm serum parametreleri ile yaş, sigara içimi, Doğu Kooperatif Onkoloji Grubu performans durumu (ECOG) ve cinsiyet arasında istatistiksel olarak anlamlı bir ilişki bulunamadı ($p > 0.05$). Ayrıca tüm ölçülen serum parametreleri ile KHDAK'li hastaların yaşam süreleri arasında anlamlı bir ilişki yoktu ($p > 0.05$). **Sonuç:** KHDAK'li hastalarda MMP-9, VEGF ve sICAM serum düzeyleri yüksek olarak bulunurken MMP-13 düzeyinde anlamlı farklılık saptanmadı. MMP-9, VEGF ve sICAM serum düzeylerinin ölçümü potansiyel bir tümör markırı olabilir.

Anahtar Kelimeler: Damar endoteli büyüme faktörleri; akciğer; matriks metalloproteinazlar; hücrelerarası yapışma molekülü-1

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Lung cancer is the most frequent cause of cancer death among malignant diseases.[1] More than one million new cases of lung cancer occur worldwide every year.[2] Among the pulmonary carcinomas, non-small cell lung cancer (NSCLC) accounts for more than 75 percent.[3] High percentage of distant metastases is determined at the time of diagnosis of NSCLC.[4,5]

Neovascularisation is a requirement for the growth and metastasis of solid tumours. There is a balance in angiogenetic process between stimulatory and inhibitory factors. Vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) are most potent two factors involved in angiogenesis.[3] Most of the studies revealed that MMPs including MMP-9, MMP-2 and MMP-13 facilitate tumor invasion, metastasis and tumor related angiogenesis.[6] Soluble intercellular adhesion molecule-1 (sICAM-1) has an important role in tumor progression and metastasis and it has an important correlation with both metastatic potential and poor prognosis.[7,8] Investigation of specific proteins released into circulation by cancer cells is a feasible approach to detect early lung cancer and metastasis.[9]

The aim of this investigation is to analyze the association between pretreatment serum levels of MMP-9, MMP-13, sICAM-1 and VEGF, and clinicopathological parameters and survival rate in patients with NSCLC, and to determine whether these proteins might be useful tumor markers for lung cancer.

MATERIAL AND METHODS

PATIENTS

Seventy two patients with non-small cell lung cancer and 46 healthy nonsmoker controls were enrolled in this prospective investigation.

For all patients, the diagnosis of lung cancer was confirmed by histological examinations of biopsy and cytologic specimens taken during bronchoscopic examinations. Staging was based on the new international staging system.[10] The staging procedure included clinical examination, chest X-rays in posterio-anterior and lateral views, a computed tomography (CT) scan of the chest, sonography or CT of upper abdomen, radionuclide bone scan, cranial CT/MR and positron emision tomography. All patients gave their informed consents before entering this study. The study was approved by the local ethical committee.

METHODS

Before and after the appropriate treatment, twenty millilitres of venous blood samples were taken and were subsequently centrifuged. The supernatant was transferred into microtubes and stored at -80^0 C until use. Serum samples were analysed for MMP-9, MMP-13, VEGF and sICAM-1 with Human MMP-9 and MMP-13 Immunoassay kit (Bender MedSystems, Vienna Biocenter 2, Vienna, Austria) and Human VEGF and sICAM-1 Immunoassay kit (Biosource International, Inc. California, USA). These assays employ a quantitative sandwich enzyme imunoassay technique.

STATISTICAL ANALYSIS

All statistical analyses was performed using SPSS version 11.5 (SPSS, Chicago, IL). All data were presented as mean ± SD. Survival curves were plotted according to the Kaplan-Meier method and Log-rank test. Student t-test, Mann-Whitney U and Kruskal-Wallis tests were used to measure the differences in the levels of adhesion molecules between groups. Paired sample test was used for comparing the levels of all these molecules before and after treatment. Spearman Pearson correlation test was used to evaluate the relation between the serum levels of these molecules and gender, age, smoking status and Eastern Cooperative Oncology Group (ECOG) performance status. A value of $p<0.05$ was considered as significant.

RESULTS

Seventy two NSCLC patients including 62 men and 10 women whit a mean age of 60.1 ± 10.9 years, and 46 healthy nonsmoker controls, including 5 women and 41 men whit a mean age of 61.1 ± 14.7 years were enrolled in the study. There was no statistically difference between the ages ($p= 0.663$) or genders ($p= 0.780$) of the patients and the control group.

Eleven patients (15%) were staged as stage I, five (7%) as stage II, 35 (49%) as stage III, and 21 (29%) as stage IV. Median survival time was 22 months. Survival of patients with an ECOG performance status (PS) 2 or more, locally advanced tumour or metastatic disease was shorter (Table 1).

Levels of MMP-9, VEGF and sICAM-1 were higher in NSCLC patients compared to control group ($p< 0.05$). There was no statistically significance for MMP-13 levels between two groups ($p> 0.05$) (Table 2).

In 19 of NSCLC patients, the levels of MMP-9, MMP-13, VEGF and sICAM-1 remeasured after treatment, and a statistically significant decrease in the levels of sICAM-1 (826.9 ± 551.5 vs 253.9 ± 113.5) and MMP-9 (5037.2 ± 2190.8 vs 3202.2 ± 828.9) ($p= 0.001$) was found while there was no difference in the levels of VEGF (184.6±213.0 vs 128.9 ± 46.6) and MMP-13 (0.53 ± 0.41 vs 1.16±2.01) before and after treatment ($p= 0.264$) (Figure 1).

sICAM-1 and VEGF levels in patients with advanced disease (stage III and IV) of NSCLC were higher compared to patients staged as early disease (stage I and II). On the other hand, there was no statistically significant difference in the levels of MMP-9 and MMP-13 between early and advanced disease patients (Table 3).

TABLE 1: Relationship between survival time and stage, ECOG PS, lymph node and distant metastases.

	N	Median survival time (month)	P value
Stage			
I/II	16	27	
III	35	24	
IV	21	6	<0.05
ECOG PS			
0	50	24	
1	14	15	
2-4	8	3	<0.05
Lymph node metastases			
Yes	53	15	
No	19	24	<0.05
Distant metastases			
Yes	51	6	
No	21	27	<0.05

ECOG PS: Eastren Cooperative Oncology Group performance status.

TABLE 2: Levels of MMP-9, MMP-13, VEGF and sICAM-1 in patients and control group.

Parameters	Patient	Control group	P value
MMP-9 (pg/ml)	5148.2±1996.2	2943.6±851.7	P<0.05
MMP-13 (ng/ml)	0.46±0.48	0.53±1.35	P>0.05
VEGF (pg/ml)	248.7±255.9	182.1±207.5	P<0.05
sICAM-1 (ng/ml)	985.0±489.4	300.5±204.1	P<0.05

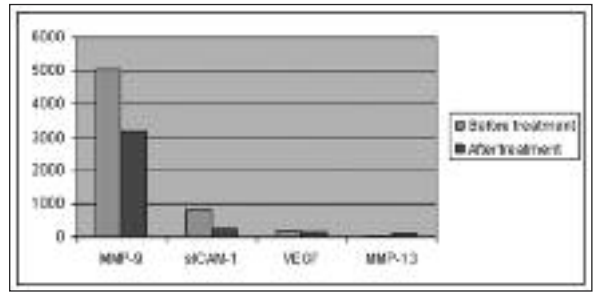

FIGURE 1: Levels of MMP-9, MMP-13, VEGF and sICAM-1 in patients with NSCLC before and after treatment.

TABLE 3: sICAM-1, VEGF, MMP-9 and MMP-13 levels according to stages of disease in patients with NSCLC.

Stage	sICAM-1	VEGF	MMP-9	MMP-13
I/II	726.3±513.7	168.2±178	5380.5±1671.5	0.56±0.41
III	1067.0±406.2	275.8±280.9	4925.9±2159.1	0.45±0.58
IV	1053.2±543	267.3±263	5320.5±2009.3	0.39±0.32

In the NSCLC patient group, there was no statistically significant difference in the levels of sICAM-1, VEGF, MMP-9 and MMP-13 with respect to age, gender, ECOG PS, the smoking status or the survival time ($P> 0.05$).

DISCUSSION

When the biology of tumor is taken into account, for the growth and metastasis of tumors, intercellular communication and nutritional support are required. Additionally, neovascularization is a requirement for solid tumour growth. Angiogenesis represents the formation of new blood vessels from the existing vasculature. Angiogenic stimulus are released by the tumor, stromal and inflammatory

cells, by the extra-cellular matrix, or by the endothelial cells.[3,9,11,12]

MMP and VEGF are two of the most potent proteins involved in angiogenesis. MMPs are extracellular matrix-degradative enzymes capable of degrading many extracellular matrix proteins. The degradation of extracellular matrix is an essential step in the spread of cancer cells. These enzymes have been implicated in tumor invasion and metastasis.[13,14] Although 18 members of the MMP family, MMP-9, MMP-2 and MMP-13 have been detected in malignant tissues and are associated with tumour aggressiveness and metastaic potential,[15-18] MMP-9 and MMP-13 are expressed in many different human tumors including lung, colon, breast and prostate carcinomas. Elevated levels of these markers have been showed in patients with gastric, colon, breast and prostate cancers.[9]

VEGF is the most potent and spesific growth factor for endothelial cells. High levels of expression of VEGF are found in many solid tumor types including colon, breast, gastric and squamous cell lung cancer.[19-22] We observed a significant elevation of serum levels of MMP-9, VEGF and sICAM-1 in lung cancer patients when compared to healty controls. Similarly, Li et al. found that VEGF might be a useful serologic biomarker for clinical diagnosis and prognosis of ovarian cancer.[23]

Hrabec et al.[9] and Kaya et al.[24] have observed that the serum levels of plasma MMP-9 in lung cancer patients were significantly higher than those of healthy subjects, however this result could not is directly interpreted as over-expression of this enzyme by cancer cells.[9]

Ondo et al. suggested that the concentration of serum active MMP-9 might be a potentially useful marker for patients with NSCLC to detect recurrent disease in the follow-up period.[25] Additionally, determination of serum angiogenetic molecules can be easily performed in samples obtained before treatment.

Cellular adhesion has an important role in intracellular and intercellular communication, and sICAM-1 has been implicated in tumor progression and metastases.[26] De vita et al. and Kamiyashihara et al. showed that the sICAM-1 concentrations in lung cancer patients played a role in staging, and also might serve as a useful indicator of advanced disease and elevated levels could be of prognostic importance in patients with NSCLC.[7,8] In our study, we observed a significant elevation of serum levels of sICAM-1 in lung cancer patients when compared to healty controls. Güney et al. determined that the level of sICAM-1 in lung cancer patients was higher than healthy subjects, but there was no difference between the level of sICAM-1 and patients characteristics and tumor staging.[26]

Laack et al. found positive correlation between tumor stage and pretreatment serum levels of both MMP-9 and VEGF.[3] In our study, there was an increased level of serum sICAM-1 and VEGF in advanced stages (stage III and IV) when compared to early stages (stage I and II) in lung cancer patients. We found no relationship between serum MMP-9, MMP-13 levels, and tumour staging. Kaya et al. found that staging, nodal status, tumor status or histology did not correlate with the circulating MMP-9 levels. However, their metastatic group had higher MMP-9 levels. So they tought that MMP-9 levels might have a prognostic value.[24] In other studies, there was no relationship between these parameters and tumor stage or metastasis.[9,11,12]

In the study of Laack et al., the pretreatment serum level of MMP-9 was identified as an independent prognostic factor and had a higher prognostic relevance than that of VEGF.[3] In most published studies, serum VEGF level has no prognostic value on survival.[11,16] In the study of Sprenger et al., there was an increase of sICAM-1 expression during the progression of disease coincided a poorer survival prognosis in the patients with stable or falling sICAM-1 levels.[27] We also found that sICAM-1 and VEGF levels were higher in patients with advanced disease when compared to patients with early disease.

Laack et al. showed a significant relationship between serum levels of MMP-9, VEGF, and survival in NSCLC patients with stage I/II.[3] Although no a significant difference in survival was observed in patients with stage III disease, they observed signi-

ficant difference between serum level of VEGF and survival in patients with stage IV disease.[3] We did not find a significant relationship between serum levels of MMP-9, MMP-13, VEGF, and survival in our study.

The prognostic value of serum levels of MMP and VEGF in patients with NSCLC is still unclear. A significant relationship between serum level of VEGF and survival was not determined in most studies.[28,29] In another study, sICAM-1 levels were higher in the lung cancer patients than the control group, but there was no difference in terms of patient characteristics and disease stage.[15] However after the appropriate treatment, sICAM-1 levels decreased. In our study, serum sICAM-1 and MMP-9 levels decreased after the treatment.

The promising findings need to be confirmed with further studies with larger patient populations since the identification of the mechanism of these proteins may lead to a better comprehension of the natural history of lung cancer and may help developing new treatment strategies.

In conclusion, serum levels of MMP-9, VEGF and sICAM-1 were higher in lung cancer patients when compared to healthy subjects, but there was no statistically significance for MMP-13 levels. In addition, the levels of MMP-9, MMP-13, VEGF and sICAM-1 were remeasured after treatment and significant decrease in the levels of sICAM-1 and MMP-9 were found while there was no difference in the levels of VEGF and MMP-13 before and after treatment. Investigation of serum levels of MMP-9, VEGF and sICAM-1 might be useful as tumor markers for lung cancer.

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