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MATRIX METALLOPROTEINASE-9 IN BRONCHO-ALVEOLAR LAVAGE FLUID OF PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Aim: **To evaluate concentration of MMP-9 in blood plasma and broncho-alveolar lavage fluid (BALF) from patients with non-small cell lung cancer (NSCLC).** ***Methods:*** **Blood plasma from 40 NSCLC patients and 40 healthy donors was collected and concentrations of blood plasma and BALF MMP-9 were measured using ELISA. Correlation between MMP-9 level and gender, histological type of tumor and stage of disease was analyzed.** ***Results:*** **Levels of blood plasma MMP-9 were significantly higher in NSCLC patients ($p < 0.0001$) then in control group, and were especially high in patients with stage IV of disease (stage I vs stage IV — $p < 0.005$, stage II vs stage IV — $p < 0.01$, stage III vs stage IV — $p < 0.01$). Also, stage IV of NSCLC was characterized by the highest level of BALF MMP-9 (stage I *vs* stage IV — $p < 0.002$, stage II *vs* stage IV $p < 0.002$, and stage III *vs* stage IV $p < 0.007$). Correlation between blood plasma and BALF MMP-9 levels and gender or histological type of tumor was insignificant.** ***Conclusion:*** **Our data revealed significant correlation between tumor stage and BALF and plasma MMP-9 levels in NSCLC patients.**

Key Words**: matrix metalloproteinase-9, plasma, broncho-alveolar lavage, nonsmall cell lung cancer, metastasis, histological type.**

Metalloproteinases (MMPs) are group of enzymes that play significant role in the metabolism of extracellular matrix components, tissue repair and cell migration [1]. MMPs are secreted in inactive form from different kind of cells or are expressed as plasma membrane bound form. MMPs play important roles in tumor invasion and metastasis and are also involved in initial stages of tumor development by regulating cell proliferation, apoptosis, angiogenesis and immune surveillance [2, 3]. Matrix metalloproteinase-9 (MMP-9) belongs to the family of gelatinases that occupy special position because of their ability to degrade both elastin and type IV collagen — major component of basal membrane [4, 5].

Lung cancer, the leading cancer type in the developed countries, has been widely investigated for its etiological factors. Some natural genetic variations have been associated with individual susceptibility to this tumor [6, 7]. More than one million new causes of lung cancer occur in the world every year. Non-small cell lung cancer (NSCLC) accounts more than 75% of lung cancers.

Elevation of MMP-9 level associated with metastasis and with decrease of survival time, has been reported [8–11]. The aim of this study was to investigate the interaction between the levels of MMP-9 in broncho-alveolar lavage fluid (BALF) and plasma of patients with NSCLC and to analize correlation between these indices and tumor stage.

Blood plasma samples from 40 healthy donors (29 males and 11 females) and 40 NSCLC patients (32 males and 8 females) cured in Ankara Numune Teaching and Research Hospital (Turkey) from March 2004 to April 2005, were studied. The mean age of NSCLC patients and controls was 56 ± 8.3 years (range 31–71) and 54 ± 10.0 years (range 34–72), respectively. Based on the WHO criteria, the patients group consisted of 14 patients with adenocarcinoma and 26 with squamous cell carcinoma. Nineteen patients had early stage of disease (6 patients with stage I and 13 patients with stage II), 13 patients had locally advanced disease (stage III), 8 patients were at stage IV. All patients were staged at the time of surgery following the guidelines of the American Joint Committee on Cancer Staging [12]. The procedure included chest radiography, computer tomography (CT) scans of the chest and abdomen, magnetic resonance imaging of the brain and bone scanning. Local ethical committee approved all protocols for this study. Patients with NSCLC and healthy donors were smokers. Clinical parameters of patients are summarized in Table 1.

Table 1. Clinicopathologic parameters of patients with NSCLC

Parameters	NSCLC patients (n = 40)
Age (years)	
Mean	56 ± 8.3
Range	31–71
Gender	
Male	32
Female	8
Histological Type	
Adenocarcinoma	14
Squamous cell carcinoma	26
Stage	
IA	1
IB	5
IIA	4
IIB	9
IIIA	9
IIIB	4
IV	8

BALF were collected only from the group of patients with NSCLC. Venous blood samples obtained from NSCLC patients and donors were placed in tubes containing EDTA, immediately centrifuged (1500 g x 10 min). BALF and plasma were stored in aliquots at –80 °C until use.

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Abbreviations used: BALF – broncho-alveolar lavage fluid; MMP-9 – matrix metalloproteinase-9; NSCLC – non-small cell lung cancer.

ELISA was performed to detect MMP-9 in serum and BALF samples, using Quantikine kits (R & D Systems Inc., Minneapolis, USA) and specific antibodies by routine procedure. The optical density was detected using Tecan Sunrise Elisa Microplate Reader (UK) at wave length of 450 nm. Each sample was assayed in duplicate and values were within the linear portion of the standard curve. The minimum detectable concentration was less than 0.156 ng/ml.

Values were expressed as Mean ± SE. The difference between groups was evaluated by Students *t*-test. The difference between stages was measured by Kruskall-Wallis and than Mann Whitney *U*-test. For correlation analysis Pearsons test was used. The difference was considered significant if $p < 0.01$.

The plasma MMP-9 level of NSCLC patients (271.87 ± 189.02 ng/ml) was significantly higher than in control group (118.80 ± 97.17 ng/ml, $p < 0{,}0001$). Level of BALF MMP-9 was determined in NSCLC patients (855.87 ± 606.39 ng/ml). Level of plasma MMP-9 from patients with stage IV was significantly higher than in controls (Table 2).

Table 2. Levels of plasma and BALF MMP-9 in patients with NSCLC.

	Sex F/M	Hystologic Type A/S	Plasma MMP-9 ng/ml	BALF MMP-9 ng/ml
Stage I	1/5	1/5	176.5 ± 90.95*	412.17 ± 108.84**
Stage II	4/9	5/8	198.38 ± 86.98	636.15 ± 334.45
Stage III	3/10	6/7	211.69 ± 83.11	780.34 ± 387.71
Stage IV	0/8	2/6	560.62 ± 220.25*	1667.62 ± 754.04**
Control	11/29	–	118.80 ± 97.17	–

Female/Male F/M, Adenocarcinoma/Squamous cell carcinoma A/S

*$p < 0.005$ stage I–IV plasma MMP-9 levels

**$p < 0.002$ stage I–IV BALF MMP-9 levels

Plasma and BALF MMP-9 levels in stages I patients were significantly lower than these in stages IV patients ($p < 0.002$ and $p < 0.005$, respectively). MMP-9 levels in BALF were similar at early stages, but that in stage IV was characterized by the highest level of BALF and plasma MMP-9 levels (stage I vs stage IV — $p < 0.002$, stage II vs stage IV — $p < 0.002$, and stage III *vs* stage IV — $p < 0.007$ for BALF, stage I vs IV — $p < 0.005$, stage II vs IV — $p < 0.01$, and stage III vs IV — $p < 0.01$ for plasma MMP-9).

The correlation between MMP-9 level in blood plasma or BALF and the stage of disease was found significant (Pearson's $r = 0.608$ ($p < 0.0001$), Pearson's $r = 0.630$ ($p < 0.0001$, respectively). However, no correlation between plasma or BALF MMP-9 levels and gender ($r = 0.032$ for plasma, $r = 0.300$ for BALF, $p > 0.05$), or histological type of tumor ($r = 0.208$ for plasma, $r = 0.079$ for BALF) has been revealed.

So, our study has demonstrated the significant increase of plasma MMP-9 level in NSCLC patients compared to healthy donors. This finding is in accordance with the data of other authors [9, 11, 13, 14] reporting on elevation of plasma or serum MMP-9 levels in NSCLC patients. In agreement with previous reports [15, 16], our results showed that MMP-9 levels do not depend significantly on patient's gender or/and histological type of tumor.

We report for the first time an association between BALF MMP-9 levels and stage of NSCLC. The levels of BALF MMP-9 are increased upon advanced lung cancer, however the mechanisms responsible for the elevation of MMP-9 levels remain unclear. Further studies on a larger number of patients are required to explore the prognostic values for these metastasis-associated markers for NSCLC.

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СОДЕРЖАНИЕ МАТРИКСНОЙ МЕТАЛЛОПРОТЕИНАЗЫ 9 В БРОНХОАЛЬВЕОЛЯРНОЙ ЖИДКОСТИ БОЛЬНЫХ НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО

Цель: **определить концентрацию матриксной металлопротеиназы 9 (ММП-9) в плазме крови и бронхо-альвеолярной жидкости (БАЖ) больных немелкоклеточным раком легкого (НМКРЛ).** ***Методы:*** **концентрацию ММП-9 в плазме крови и БАЖ больных НМКРЛ (n = 40) и здоровых доноров (n = 40) определяли иммуноферментным методом и анализировали корреляцию этих параметров с клиническими данными (полом больного, гистологическим типом опухоли, стадией заболевания).** ***Результаты*****: содержание ММП-9 в плазме крови было значительно выше у больных НМКРЛ по сравнению с контрольной группой ($p < 0{,}0001$), особенно у больных с IV стадией заболевания. Стадия IV НМКРЛ также характеризовалась наиболее высоким уровнем ММП-9 в БАЖ. Корреляции между уровнем ММП-9 в плазме крови и БАЖ, полом больного и гистологическим типом опухоли не была выявлена.** ***Выводы:*** **существует статистически значимая корреляция между стадией развития НМКРЛ и содержанием ММП-9 в плазме крови и БАЖ больных.**

Ключевые слова**: матриксная металлопротеиназа 9, плазма крови, бронхо-альвеолярная жидкость, немелкоклеточный рак легкого, метастазы, гистологический тип опухоли.**