The evaluation of soluble Fas and soluble Fas ligand levels of bronchoalveolar lavage fluid in lung cancer patients

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ÖZET

Akciğer kanserli hastalarda bronkoalveoler lavaj sıvısında çözünebilir Fas ve Fas ligand düzeylerinin belirlenmesi

Fas-Fas Ligand (FasL), programlanmış hücre ölümünü aktive eden majör mediatör sistemlerden biridir. Membrana bağlı FasL'nin metalloproteinaz grubu bir enzimle reaksiyona girmesi sonucu çözünebilir FasL (çFasL) oluşur. çFasL ile birlikte FasL'nin transmembran formu Fas'a bağlanır ve apopitotik uyaranları Fas belirteci taşıyan hücrelere sunar. Çözünebilir Fas (çFas) ve çFasL'nin tümör gelişiminde ve tümör hücrelerinin konağın immün sisteminden kaçmasında rolü olduğu ileri sürülmüştür. Akciğerlerdeki Fas antijen belirteçlerinin alveol ve bronş epitel hücresinde lokalize olması nedeniyle bu çalışmada, akciğer kanserli hastaların bronkoalveoler lavaj (BAL) sıvısında çFas (pg/mL) ve çFasL (pg/mL) düzeylerini belirlemeyi amaçladık. Çalışma popülasyonunu akciğer kanserli 27 hasta (ortalama yaş 62.9 ± 10.7 yıl) ve 25 kontrol olgusu (ortalama yaş 47.9 ± 13.9 yıl) oluşturmaktaydı. BAL işlemi lokal anestezi altında olguların hastalıktan etkilenmemiş olan akciğerinde, lingulanın veya sağ akciğer orta lobun subsegmentlerinden yapıldı. BAL sıvısı çFas ve çFasL düzeyleri ELISA metodu kullanılarak ölçüldü. BAL sıvısı ortalama çFas düzeyleri akciğer kanserli hastalarda 60.8 ± 56.8 ve kontrol olgularda 39.5 ± 25.9 olarak bulundu (p> 0.05). BAL sıvısı ortalama çFasL düzeyleri kanserli hastalarda 51.6 ± 39.2, kontrol olgularda 41.2 ± 27.4 olarak saptandı (p> 0.05). Sonuç olarak her iki grup arasında anlamlı bir farklılık gözlememekle birlikte, çFas ve çFasL düzeylerinin akciğer kanserli hastaların BAL sıvısında kontrol olgulara göre yüksek saptanması akciğer kanserinin oluşmasında ve ilerlemesinde apopitozisin bir rolü olabileceği kanısına vardırdı.

Anahtar Kelimeler: *Akciğer kanseri, bronkoalveoler lavaj, çözünebilir Fas, çözünebilir FasL.*

SUMMARY

The evaluation of soluble Fas and soluble Fas ligand levels of bronchoalveolar lavage fluid in lung cancer patients

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Fas-Fas Ligand (FasL) is one of the major mediator system that activates programmed cell death. Cleavage of membrane-bound FasL by a metalloproteinase-like enzyme resulted in the formation of soluble FasL (sFasL). sFasL as well as the transmembrane form of FasL binds to Fas and transduces apoptotic signal in Fas-expressing cells. It's suggested that soluble Fas (sFas) and sFasL has an impact on tumor progress and immune escape feature of tumor cells from the host immune system. Since Fas antigen expression in the lungs has been localized to alveolar and bronchial epithelial cells, in this study we aimed to investigate the sFas (pg/mL) and sFasL levels (pg/mL) of bronchoalveolar lavage (BAL) fluid in lung cancer patients. Study population was consisted of 27 patients with lung cancer (mean age 62.9 ± 10.7 years, 25 control subjects (mean age 47.9 ± 13.9 years). BAL was performed under local anesthesia, on the unaffected lung of patients; either subsegments of right middle or lingula. BAL sFas and sFasL were evaluated by using ELISA method. The mean levels of sFas was 60.8 ± 56.8 in lung cancer patient and 39.5 ± 25.9 in control subjects (p> 0.05). The mean levels of sFasL was 51.6 ± 39.2 in cancer patient and 41.2 ± 27.4 in control subjects (p> 0.05). In conclusion, although we did not observe any significant difference between two groups, higher BAL levels of sFas and sFasL levels in lung cancer patients than control subjects, made us thought that apoptosis might have a role development and progression of lung cancer.

Key Words: *Lung cancer, bronchoalveolar lavage, sFas, sFasL.*

Apoptosis was described as programmed cell death and is one of the most important regulatory mechanisms of cellular homeostasis (1). Fas is a receptor which transmits an intracellular signal that leads to programmed cell death through binding with its ligand (FasL). Fas (APO-1, CD95) and Fas Ligand (FasL) have been known as transmembrane proteines and member of the tumor necrosis factor receptor family (2). In contrast to expression of Fas in majority of the cells, the expression of FasL in normal tissues is limited to activated T-lymphocytes, natural killers cells, and to a few immuno priviliged tissues such as the brain, eye, testis and placenta (3-8). Immuno privilege is a unique trick that is executed by constitutive expression of Fas to avoid inflammatory reaction, one that has been adopted by specialized organs including the testis, brain and corneal tissues (6).

Recent studies have shown that, ovarian carcinoma, colon carcinoma, hepatocellular carcinoma, liver metastasis of colon carcinoma cells, brain tumors, melanoma and lung carcinoma also express FasL that may trigger apoptosis of activated lymphocytes (9-15). This process propose that the FasL may offer a survival advantage to tumors. It was also suggested that membrane-bound FasL, cleaved by a specific matrix metalloproteinase-like enzyme and be present in a soluble form (16). Human soluble FasL is a 26-kilodalton glycoprotein consisting of an extracellular region for binding with Fas and is produced by activated T-cells (17,18). Serum concentrations of soluble FasL (sFasL) have been noted in patients with natural killer cell lymphoma, nonhematopoetic malignancy, hepatocellular carcinoma and graft-versus-host disease (19-22). The objective of this study was to investigate the role local apoptosis markers in lung cancer by determining bronchoalveolar lavage (BAL) fluid concentration of sFas and sFasL in patients with lung carcinoma.

MATERIALS and METHODS

Study Population

We studied 27 untreated patients with lung cancer, representing all patients with this disease who were admitted to our clinic between 2002 and 2003. They presented with radiographic abnormalities suggestive of lung cancer and the diagnosis of lung cancer was confirmed with following histological examination of biopsy samples from the lungs. They included 27 males and no female, ranging in age from 40 to 86, with a median age of 62.9 ± 10.7 years.

The control group was consisted of 25 healthy subjects (14 males 11 females, ranging in age from 21 to 53 years, with a median age of 47.9 ± 13.9 years) who had no previous history of pulmonary diseases or airway infection. BAL has been done due to various reasons (hemoptysis, atelectasis). The study was approved by the local ethics committee and each individual gave informed consent.

Bronchoalveolar Lavage (BAL)

BAL was performed using a standard technique. The patient was premedicated intramuscularly with atropine (0.5 mg). After local anesthesia with 2% lidocaine, a flexible bronchoscope was wedged into either subsegments of right middle or lingula for lavage on the unaffected lung of patients. Sterile physiological saline solution at body temperature was instilled through the bronschoscope and the fluid was immediately retrieved by gentle suction using a sterile syringe. The collected lavage fluid was filtrated through two sheeds of gauze and centrifuged at 400 g for 10 min at 4°C and the supernatant was stored at -80°C until use.

Measurement of sFasL and sFas

sFas and sFasL levels were measured in BAL fluid samples by the solid-based sandwich ELISA kits following the instructions of manufacturer (Diaclone, Cedex, France). The absorbance was determined using an ELISA reader at 450 nm. The concentrations of sFas and sFasL were determined from a calibration curve constructed using a reference standard.

Statistical Analysis

All values were expressed as medians (with ranges). The Mann-Whitney U test was used to compare differences between two groups. A p value of 0.05 was accepted us a statistically significant.

RESULTS

The characteristics of patients with cancer and control patients are summarized in Table 1.

The mean levels of sFas was 60.8 ± 56.8 pg/mL in lung cancer patient and 39.5 ± 25.9 pg/mL in control subjects ($p > 0.05$) ($p = 0.349$) but the difference was not statistically significant (Figure 1). The mean levels of sFasL was 51.6 ± 39.2 pg/mL in cancer patient and 41.2 ± 27.4 pg/mL in control subjects ($p > 0.05$) ($p = 0.341$) (Figure 2). Also this difference was not statistically significant.

DISCUSSION

Fas mediated cell death has shown to be an important factor in cancer cell elimination (23). We found higher levels of sFas and sFasL in BAL fluids of patients with cancer but these levels were not significantly higher than the control subjects.

Table 1. Characteristics of the patients and control group.

	Malign group	Control subjects
Age (year)	62.9 ± 10.7*	47.9 ± 13.9*
Gender (M/F)	27/0	14/11
Tobacco consumption (pack/year)	45.7 ± 24.5*	16.3 ± 28*

* p= 0.000

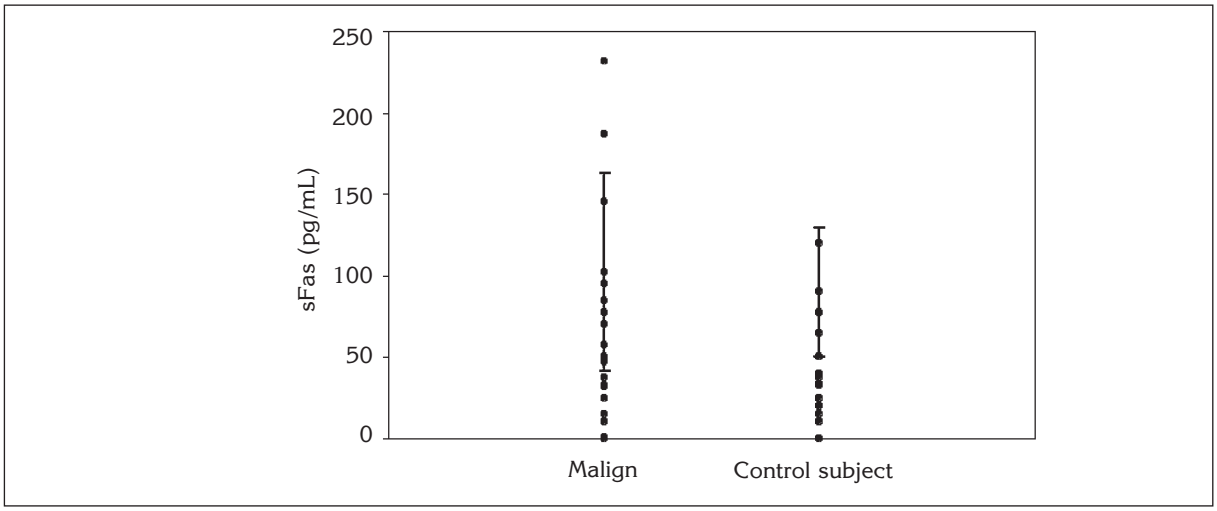

Figure 1. BAL sFas levels in patients with lung cancer and control subjects.

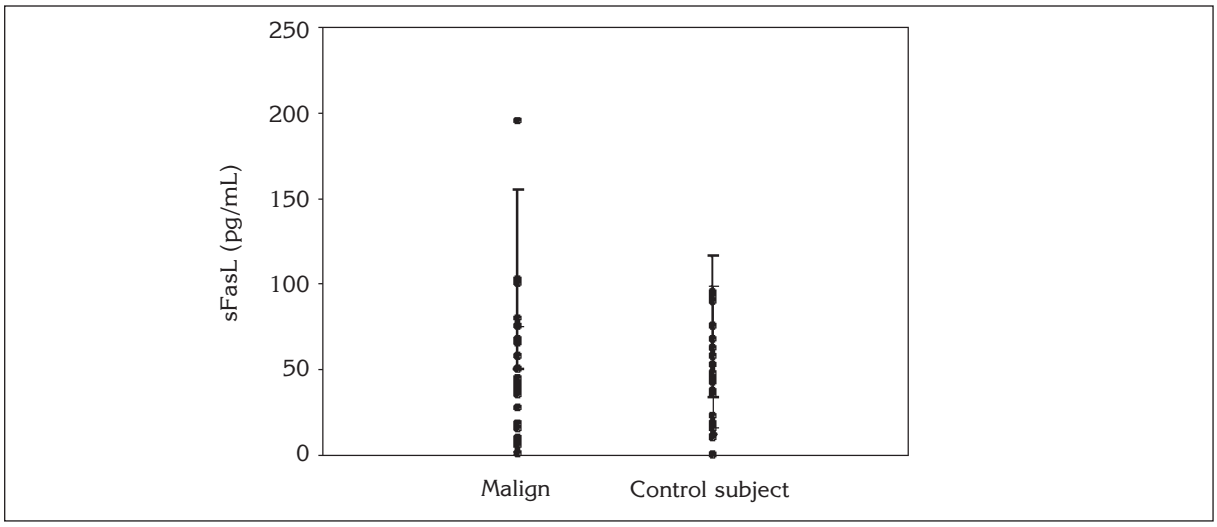

Figure 2. BAL sFasL levels in patients with lung cancer and control subjects.

Serum sFasL, sFas concentrations have been assessed in patients with various lung disease, such as fibrosing lung diseases and silicosis and lung carcinoma and different carcinomas such as adrenal tumors, bladder cancer and colon cancer (24-29). BAL fluid concentrations of sFasL, sFas have been measured only bronchiolitis obliterans organizing pneumonia, hypersensitivity pneumonitis, acute respiratory distress syndrome (ARDS) and sarcoidosis (30-33). The present study is the first one, investigating the sFas and sFasL in BAL fluid of lung cancer patients.

Different studies demostrated by Fas and FasL expression in tumor tissue of patient with lung cancer using immunohistochemistry (34,35). Niehans et al demonstrated that all human lung carcinoma cell lines express FasL by using immunoblotting method (15). Yoshihiro reported that 2 of 4 bronchioloalveolar cell carcinomas, and 20 of 42 total pulmonary adenocarcinomas expressed Fas but sFas was not detected in the majority of these tumors either by Proof-reading Polymerase Chain Reaction (RT-PCR) or Western blot analysis (34). Yoshimura et al. analyzed serum sFas and sFasL expression by ELISA in patients with non-small cell lung cancer and found higher levels of sFas and sFasL in serum of patients with lung cancer than the control subjects (36).

Koomagi reported that lung cancer patients with Fas-positive tumors exhibited significantly longer survival times than patients with Fas-negative carcinomas. But FasL did not significantly influence patient's survival time in their study (35). In contrast, Yoshihiro did not find significant correlation between Fas protein expression and prognosis of lung cancer patients (34). We did not investigate the correlation between BAL sFas and sFasL levels of lung cancer patients with their survival.

In conclusion, although we did not observe any significant difference, higher BAL levels of sFas and sFasL in lung cancer patients than control subjects, made us thought that apoptosis might have a role development and progression of lung cancer.

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