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Analysis of insulin like growth factor 1 and insulin like growth factor binding protein 3 levels in bronchoalveolar lavage fluid and serum of patients with lung cancer

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Summary *Objective*: Insulin like growth factor 1 (IGF-1) is recognized as a potent mitogen for many cancer cell lines and there is good evidence that lung cancer cells produce both IGF-1 and insulin like growth factor binding protein 3 (IGFBP-3). The aim of this study was to investigate the clinical significance of IGF-1 and IGFBP-3 levels in serum and in bronchoalveolar lavage (BAL) fluid by comparing lung cancer patients with healthy controls.

Methods: BAL fluid and serum samples were obtained from 24 lung cancer patients and 12 healthy controls, and were analyzed for IGF-1 and IGFBP-3 levels by a two site immunoradiometric assay. The recovered BAL fluid was standardized by albumin to remove the variable of dilution and the data was expressed in epithelial lining fluid (ELF).

Results: Serum IGF-1 and IGFBP-3 levels were lower in lung cancer patients, but the difference between the groups did not reach a statistical significance. IGF-1/IGFBP-3 ratio in ELF was significantly lower in lung cancer patients ($P = 0.035$). Mean IGF-1 level in ELF was determined to be significantly lower in patients with distant metastasis ($P = 0.04$). Serum IGF-1/IGFBP-3 ratio was found to be significantly lower in patients with distant ($P = 0.04$) and nodal metastasis ($P = 0.03$). Tumor stage was negatively correlated with IGF-1 level in ELF ($P = 0.05$, $r = -0.4$) and serum IGF-1/IGFBP-3 ratio ($P = 0.04$, $r = -0.4$).

Conclusion: IGF-1 and IGFBP-3 levels both in serum and ELF might serve a clinical significance in patients with lung cancer. However, further studies comprising more

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cases are needed to investigate the clinical significance of IGF-1 and IGFBP-3 in lung cancer.

Introduction

Insulin like growth factors (IGFs) are important mitogens acting as endocrine, paracrine and autocrine agents.[1,2] They are present in most of the biological fluids and carried by specific binding proteins (IGFBPs). The major form of binding protein known in human circulation is IGFBP-3, which modulates IGF activity and also regulates cell growth and apoptosis.[1,3] IGF-1 is recognized as a potent mitogen for many cancer cell lines and plays an important role in neoplastic transformation.[1–5] In the recent epidemiologic studies, the association between IGFs and prostate cancer, breast cancer, colorectal cancer and also lung cancer has been demonstrated.[2,6–9]

There is a good evidence that lung cancer cells produce both IGF-1 and IGFBP-3 and the circulating levels of IGF-1 and IGFBP-3 may be important in determining the risk of lung cancer.[5,9] Also, previous studies have examined the serum levels of IGF-1 and IGFBP-3 in lung cancer patients and the relationship with the stage of disease and histopathologic type.[10,11] This is the first study investigating the clinical significance of IGF-1 and IGFBP-3 levels in serum and in bronchoalveolar lavage (BAL) fluid by comparing lung cancer patients with healthy controls.

Materials and methods

Patients

The study was conducted on 36 subjects who were divided into two groups. Group 1 consisted of 24 lung cancer patients. The diagnosis of malignancy was confirmed by bronchial lavage, sputum cytology or tissue biopsy specimens. Group 2 consisted of 12 control subjects who had undergone fiberoptic bronchoscopy (FOB) for various reasons (7 for hemoptysis work up, 5 for non-productive cough) and no pathologic finding could be reached eventually.

Patients with the diseases affecting the serum levels of IGF-1 such as inflammatory bowel diseases, chronic liver disease, renal failure, hepatocellular carcinoma and interstitial lung disease were excluded from the study. Women receiving hormone replacement therapy were also not candidates for our study since hormone replacement therapy increases serum IGF-1 levels. The study was approved by the Local Ethics Committee of Atatürk Chest Diseases and Chest Surgery Research Hospital and informed consent was obtained from all the subjects enrolled in the study.

FOB and BAL

A patient with a pulmonary mass lesion was a candidate for our study. At the time of diagnostic bronchoscopy we collected BAL from lobar or segmental bronchus of the disease side. The patients with endobronchial lesions occluding the bronchus and hemorrhagic BAL were excluded from the study.

All subjects were premedicated subcutaneously with atropine (0.5 mg) and intramuscular diazepam (5 mg). The upper respiratory tract was anesthetized topically with 2% prilocaine via an ultrasonic nebulizer. Bronchoscopy was performed with a flexible FOB. BAL was accomplished through the FOB by instillation of sterile 0.9% saline solution (three times 50 ml aliquots) into the bronchoalveolar tree, bronchus of the disease side in lung cancer patients, right middle lobe or lingula in control subjects.

The BAL fluid was retrieved immediately with 'zero dwell time' by gentle suctioning. The mean recovery of the injected fluid was similar in groups 1 and 2 and found to be $31 \pm 11.3\%$ and $33.5 \pm 7.6\%$, respectively. The collected fluid filtered through three layers of sterile gauze and then centrifuged at 800 rpm for 10 min. After centrifuging the supernatant was divided into two, one for IGF-1, IGFBP-3 and one for albumin determination and stored at $-40\,^{\circ}C$ for later analysis.

Blood samples

As there is a circadian rhythm of IGF-1 secretion, we collected the blood samples from each case between 9:00 and 10:00 o'clock in the morning, the day after the bronchoscopy. Ten milliliter of venous blood sample was drawn from every patient, centrifuged immediately at 1500 rpm for 10 min. The serum was divided into two, one for IGF-1, IGFBP-3 and one for albumin determination and stored at $-40\,^{\circ}C$ for later analysis.

Analysis of IGF-1, IGFBP-3 and albumin levels in BAL and serum

IGF-1 levels were measured in BAL and serum with DSL-5600 ACTIVE IGF-1 Coated Tube IRMA Kit (Diagnostic Systems Laboratories, Webster, Texas, USA), which includes acid-ethanol extraction step to separate IGF-1 from its binding protein. The procedure employed a two site immunoradiometric assay (IRMA) principle first described by Milles et al.[12] IGFBP-3 levels were measured with DSL-6600 ACTIVE IGFBP-3 Coated Tube IRMA Kit (Diagnostic Systems Laboratories, Webster, Texas, USA). The IRMA is a non-competitive assay in which the analyte to be measured is 'sandwiched' between two antibodies. The first antibody is immobilized to the inside wall of the tubes, the other antibody is radiolabeled for detection. BAL and serum albumin levels were measured by Beckman Coulter Synchron LX20 Oto Analyzer by the Bromkresolpurpur (BCP) colorimetry method.

Standardization of BAL fluid

The technique of BAL is based on the concept that aliquots of sterile saline solution infused through the bronchoscope mix with epithelial lining fluid (ELF). Since the recovered BAL fluid is a variable mixture of saline solution, ELF and ELF components, it has been difficult to estimate the actual concentration of recovered molecules in the ELF in situ. The standardization method removes the variable of dilution and allows comparison between data in different subjects and from different investigations. Standardization of IGF-1 and IGFBP-3 levels in BAL fluid with the corresponding albumin levels was done using the following formulas:[13]

(1) $ELF_{volume}=(BAL_{albumin} \times BAL_{volume})/Serum_{albumin}$.
(2) $ELF \times _{substance}=(BAL_{volume} \times BAL \times _{substance})/ELF_{volume}$.
(3) $ELF \times _{substance}=(BAL \times _{substance} \times Serum_{albumin})/BAL_{albumin}$.

Statistical analysis

Statistical analysis was carried out using SPSS program version 10.0. Results were expressed as mean $\pm$ SD. Differences between groups were evaluated by using the Mann Whitney *U*-test and differences in gender between groups were examined by χ^2-test. The Spearman rank correlation coefficient test was used to examine the association between variables. Any *P* value less than or equal to 0.05 was considered statistically significant.

Results

The sex distribution, mean ages, smoking history and body mass indexes of groups 1 and 2 are listed in Table 1. Group 1 consisted of 21 males and 3 females with a mean age of 60 $\pm$ 9 years. Group 2 consisted of 11 males and 1 female with a mean age of 43 $\pm$ 11 years. Mean smoking history of groups 1 and 2 were 56 $\pm$ 45 and 48 $\pm$ 20 pack-years, respectively. Mean body mass indexes of groups 1 and 2 were 23.4 $\pm$ 1.7 and 23.6 $\pm$ 1.6 kg/m^2, respectively. The two groups were similar in sex, smoking history and body mass indexes, but group 2 was younger than group 1 ($P = 0.0$). The histological diagnosis 24 lung cancer patients were 8 adenocarcinoma, 7 small cell carcinoma, 4 non-small cell carcinoma, 3 squamous cell carcinoma, 1 adenosquamous carcinoma, and 1 malign epithelial carcinoma. Tumor (T), node (N), metastasis (M) stages, localizations and mean tumor diameter of group 1 patients are listed in Table 2.

IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF of both groups are listed in Table 3. Serum levels of IGF-1 and IGFBP-3 were 126.9 $\pm$ 63.4 and 2277.6 $\pm$ 614.0 ng/ml, respectively, in group 1 and 167.6 $\pm$ 56.5 and 2874.7 $\pm$ 861.9 ng/ml, respectively, in group 2. Serum IGF-1 and IGFBP-3 levels were lower in lung cancer patients, but the difference between the groups did not

Table 1 The sex distribution, mean ages, smoking history and body mass indexes of the study groups.

Variable	Group 1 ($n = 24$)	Group 2 ($n = 12$)	*P*
Sex (male/female)	21 M/3 F	11 M/1 F	1.0
Mean ages (years)	60 $\pm$ 9	43 $\pm$ 11	0.0*
Smoking history (pack-years)	56 $\pm$ 45	48 $\pm$ 20	0.5
Body mass index (kg/m^2)	23.4 $\pm$ 1.7	23.6 $\pm$ 1.6	0.6

Group 1: lung cancer patients, Group 2: control subjects.
*Statistically significant.

reach a statistical significance. ELF levels of IGF-1 and IGFBP-3 were 3157.6±3919.0 and 1272.0±680.1 ng/ml, respectively, in group 1 and 4456.8±4850.8 and 1048.5±867.5 ng/ml, respectively, in group 2. ELF levels of IGF-1 and IGFBP-3 were not statistically different between the groups. IGF-1/IGFBP-3 ratio in ELF was significantly lower in group 1 (2.8±3.8) than in group 2 (5.1±6.5) ($P = 0.035$), whereas serum IGF-1/IGFBP-3 ratio was not statistically different between the two groups (0.05±0.02 vs. 0.06±0.02) ($P > 0.05$). Median and interquartile range of serum and ELF IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in groups 1 and 2 are shown in Fig. 1.

The relationship of clinicopathologic features with IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF of lung cancer patients is shown in Table 4. When the patients were grouped on the basis of tumor diameter (greater than 5 and smaller than 6 cm), histological type (non-small cell lung cancer and small cell lung cancer), and localization of tumor (central and peripheric), there was no significant difference between the groups for IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF ($P > 0.05$). When the lung cancer patients were grouped based on the presence of distant metastasis, IGF-1 level in ELF was determined to be significantly lower in patients with distant metastasis ($P = 0.04$). Serum IGF-1/IGFBP-3 ratio was found to be significantly lower in patients with distant metastasis ($P = 0.04$) and nodal metastasis ($P = 0.03$).

The correlations between the tumor diameter (cm), stages (1–4) and IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF are listed in Table 5. Tumor stage was negatively correlated with IGF-1 level in ELF ($P = 0.05$, $r = -0.4$) and serum IGF-1/IGFBP-3 ratio ($P = 0.04$, $r = -0.4$).

Table 2 Tumor (T), node (N), metastasis (M) stages, localizations and mean tumor diameter of group 1 patients.

Variables	Data
T1	1 (4.2%)
T2	5 (20.8%)
T3	7 (29.2%)
T4	11 (45.8%)
N0	7 (29.2%)
N1	2 (8.3%)
N2	7 (29.2%)
N3	8 (33.3%)
M0	11 (45.8%)
M1	13 (54.2%)
Stage 1B	1 (4.2%)
Stage 2B	2 (8.3%)
Stage 3A	3 (12.5%)
Stage 3B	5 (20.8%)
Stage 4	13 (54.2%)
Central localization	13 (54.2%)
Peripheric localization	11 (45.8%)
Mean tumor diameter	6.0±2.0 l cm (min 2 cm–max 10 cm)

Discussion

Immunoreactive IGF-1 is produced in vitro by human fetal lung explants and cultured alveolar macrophages. IGF-1 is detectable in primary lung tumor tissue at higher levels than in normal lung tissue. Thus, lung cancer cells synthesize both IGF-1 and IGFBP-3 which appear to be important factors in neoplastic transformation and metastasis.[1,5]

As tissue IGF bioactivity appears to be regulated in parallel with circulating IGF-1 level, there is a hypothesis that circulating levels of IGF-1 and IGFBP-3 may be important in determining risk of lung cancer.[2,14] Yu et al. examined 208 lung cancer patients and 218 control subjects in a case control study and they found high plasma level of IGF-1 and low plasma level of IGFBP-3 associated with increased risk of lung cancer.[9] However, in another case control study, London et al. found no association between serum IGF-1 level and lung cancer

Table 3 IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF of the study groups.

Variable	Group 1	Group 2	*P*
Serum IGF-1 (ng/ml)	126.9±63.4 (95% CI: 100.1–153.7)	167.6±56.5 (95% CI: 131.7–203.6)	0.07
Serum IGFBP-3 (ng/ml)	2277.6±614.0 (95% CI: 2018.1–2536.9)	2874.7±861.9 (95% CI: 2327–3422.3)	0.06
Serum IGF-1/IGFBP-3	0.05±0.02 (95% CI: 0.045–0.062)	0.06±0.02 (95% CI: 0.048–0.073)	0.2
ELF IGF-1 (ng/ml)	3157.6±3119.0 (95% CI: 1502.7–4812.5)	4456.8±4850.8 (95% CI: 1374.8–7538.9)	0.2
ELF IGFBP-3 (ng/ml)	1272.0±680.1 (95% CI: 984.8–1559.1)	1048.5±867.5 (95% CI: 497.3–1599.7)	0.2
ELF IGF-1/IGFBP-3	2.8±3.8 (95% CI: 1.2–4.4)	5.1±6.5 (95% CI: 0.9–9.2)	0.035*

Group 1: lung cancer patients, Group 2: control subjects.
*Statistically significant.

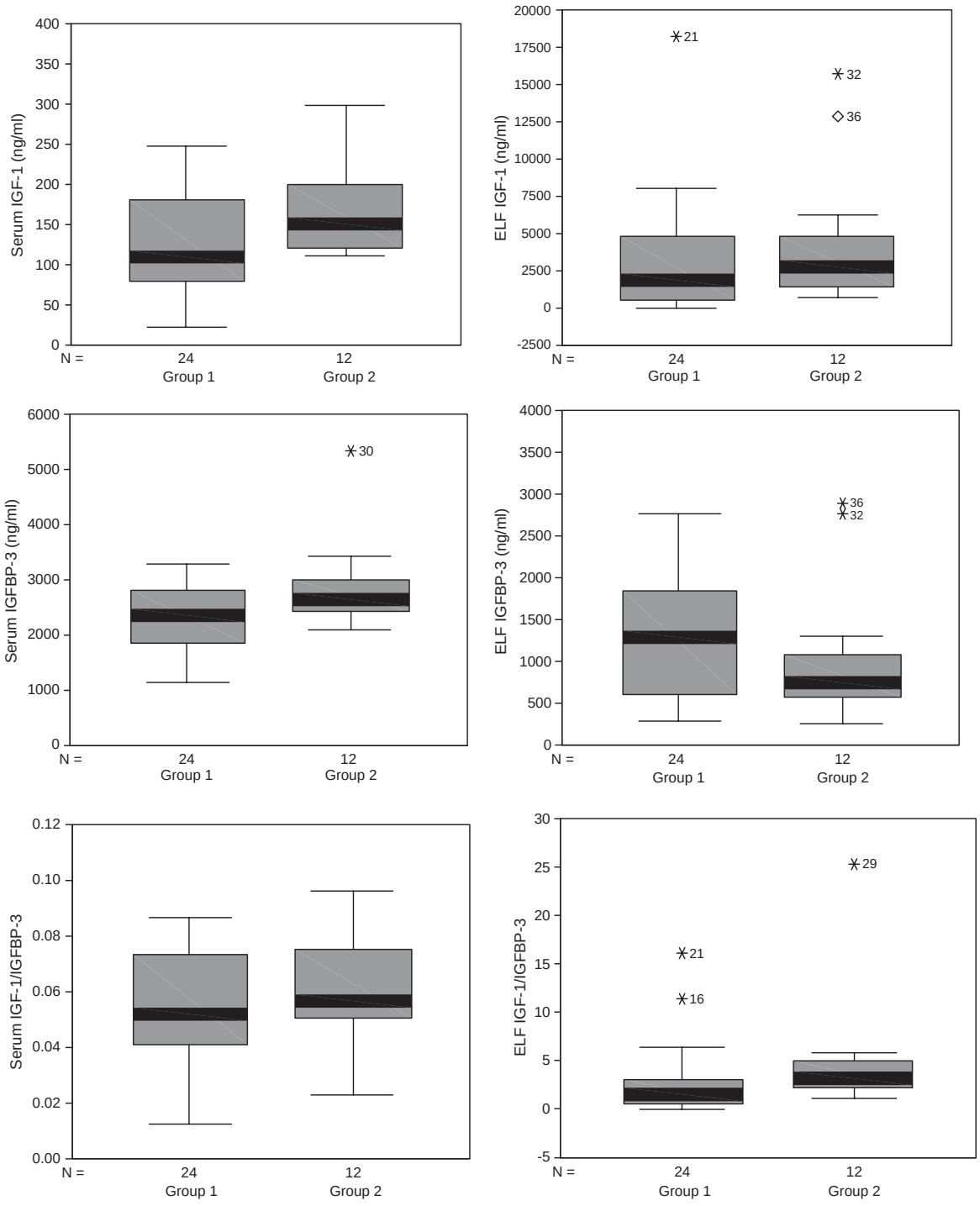

Figure 1 Median and interquartile range of serum and ELF IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in groups 1 and 2.

risk; they reported only high serum level of IGFBP-3 associated with reduced risk of lung cancer.[15]

IGFs are present in most of the biological fluids. Olchovsky et al. examined IGFs and IGFBPs levels in pleural effusion with different etiologies and IGF-1 and IGFBP-2 levels were found elevated in the group with malignant tumors. They suggested that there is a local production of IGF-1 by tumor cells and reported IGF-1 to be used in assessment of pleural effusion as a tumor marker.[16] To our knowledge, this is the first study investigating the clinical significance of IGF-1 and IGFBP-3 levels both in BAL fluid and serum of lung cancer patients by comparing them with healthy controls.

Table 4 The relationship of clinicopathologic features with IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF of lung cancer patients.

Variables	Serum			ELF		
	IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	IGF-1/ IGFBP-3	IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	IGF-1/ IGFBP-3
T. diameter						
>5 cm ($n = 10$)	115.7±62.8	2128.7±592.4	0.052±0.019	3480.2±5562.7	1240.2±790.6	3.1±4.9
<5 cm ($n = 11$)	119.9±63.4	2260.2±641.1	0.053±0.023	2776.7±1984.4	1161.2±621.2	2.8±3.1
P	0.7	0.8	0.8	0.4	0.9	0.6
N0,1 ($n = 9$)	150.8±44.5	2376.0±645.5	0.065±0.016	4202.9±5579.0	1309.1±795.0	3.5±4.9
N2,3 ($n = 15$)	112.5±70.0	2218.6±609.4	0.048±0.021	2530.4±2514.4	1249.7±630.2	2.4±3.0
P	0.09	0.6	0.03*	0.5	1.0	0.6
M0 ($n = 11$)	150.7±60.5	2356.8±577.5	0.063±0.016	4417.5±4480.8	1474.2±652.2	4.1±5.0
M1 ($n = 13$)	106.7±60.8	2210.7±658.9	0.047±0.021	2091.6±2626.7	1100.8±680.3	1.7±1.9
P	0.055	0.6	0.04*	0.04*	0.1	0.1
NSCLC ($n = 16$)	134±53.7	2381.3±611.1	0.057±0.018	3288±4469.6	1382.9±701.3	2.6±4.0
SCLC ($n = 7$)	106.6±86.5	1975.3±584.5	0.048±0.027	2872.7±2964.8	1040.4±666.3	3.1±3.8
P	0.2	0.1	0.3	0.8	0.2	0.5
Central ($n = 13$)	110.7±56.7	2245.4±673.2	0.048±0.016	4316.2±4938.2	1243.4±697.0	3.9±4.7
Peripheric ($n = 11$)	145.9±68.3	2315.8±566.0	0.062±0.023	1788.4±1501.5	1305.7±691.7	1.5±1.4
P	0.2	0.8	0.06	0.2	0.9	0.1

T: tumor, N: nodal metastasis, M: distant metastasis, NSCLC: non-small cell lung cancer, SCLC: small cell lung cancer.
*Statistically significant.

Table 5 The correlations between the tumor diameter (cm), stages (1,2,3,4) and IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 ratios in serum and ELF.

Variables	Tumor diameter (cm)	Stages (1–4)
Serum IGF-1 (ng/ml)	$r = 0.02$	$r = -0.39$
	$P = 0.9$	$P = 0.058$
Serum IGFBP-3 (ng/ml)	$r = -0.13$	$r = -0.13$
	$P = 0.5$	$P = 0.5$
Serum IGF-1/IGFBP-3	$r = 0.1$	$r = -0.4$
	$P = 0.5$	$P = 0.04$*
ELF IGF-1 (ng/ml)	$r = 0.05$	$r = -0.4$
	$P = 0.8$	$P = 0.05$*
ELF IGFBP-3 (ng/ml)	$r = 0.005$	$r = -0.3$
	$P = 0.3$	$P = 0.06$
ELF IGF-1/IGFBP-3	$r = 0.07$	$r = -0.2$
	$P = 0.7$	$P = 0.2$

*Statistically significant.

In previous studies IGF-1 levels were found to be reduced in serum of lung cancer patients which might be due to poor nutritional status or abnormal liver function.[10,11] In our study, serum IGF-1 and IGFBP-3 levels were found to be lower in lung cancer patients, but the difference between the groups did not reach a statistical significance. It is known that serum levels of IGF-1 and IGFBP-3 depend on age, smoking history and nutritional status.[17,18] In our study, two groups were similar in smoking history and body mass indexes. Both groups had a mean age over 40 years, but because of the difficulty in finding elder healthy controls, there was a statistically significant difference between the groups in age. The control group was younger than the lung cancer group. So, here it is important to recall that the difference might be due to the difference of mean ages between the groups.

IGFs system has been reported to play an important role in chronic interstitial fibrotic diseases and increased levels of IGF-1 and IGFBP-3 have been reported in the BAL fluid of patients with idiopathic pulmonary fibrosis.[19–21] But less is known about IGF/IGFBP system in BAL fluid of lung cancer patients. In our study, we did not find significant differences between lung cancer patients and the healthy controls for both IGF-1 and IGFBP-3 levels in ELF. On the other hand, IGF-1/IGFBP-3 ratio in ELF was found to be significantly lower in lung cancer patients. The alterations in IGF-1/IGFBP-3 balance have been shown to play a role in carcinogenesis.[4] Our results might be due to the interaction between IGF-1 and IGFBP-3.

Biosynthesis of IGF-1 in liver is stimulated by growth hormone (GH) and altered regulation of GH/IGF-1 system has been reported to play a role in the progression of lung cancer. Mazzoccoli et al. found that serum IGF-1 levels were lower in cancer patients, especially in advanced stages of the disease.[10] In our study, IGF-1 levels in ELF were significantly lower in lung cancer patients with distant metastasis. Also, a negative correlation was found between IGF-1 levels in ELF and the stage of the disease. This might be due to the reduced synthesis of IGF-1 in the liver especially in advanced stage of the disease.

Serum IGF-1/IGFBP-3 ratio was significantly lower in lung cancer patients with nodal and distant metastasis. Although IGF-1/IGFBP-3 ratio in ELF did not show any significant correlation with the stage of lung cancer, IGF-1/IGFBP-3 ratio in serum was found to be negatively correlated with the stage. It has been known that IGFBP-3 regulates IGF-1 activity and also cell growth and apoptosis.[1,3] There is a negative correlation between serum IGFBP-3 levels and prostate, lung, colon cancer risk and this suggests a protective role of IGFBP-3 against the effects of systemic IGF-1.[4,9] Thus, in lung cancer the balance between IGF-1and IGFBP-3 might be important in the progression of the disease. The mechanism of this is unclear and it should be investigated by further studies.

Lee et al. found that serum IGF-1 levels were lower in NSCLC patients than in the SCLC group. They reported that serum levels of IGF-1 and IGFBPs might be useful markers for diagnosing and identifying tumor types in lung cancer.[11] In contrast to this finding, we did not find any significant relationship between IGF-1, IGFBP-3 levels and histopathologic type both in serum and ELF. Also there was no relation between the tumor localization, diameter and IGF-1, IGFBP-3 levels in serum and ELF.

As a conclusion, analysis of IGF-1 and IGFBP-3 levels both in serum and ELF might serve a diagnostic value in lung cancer patients especially in the advanced stage of the disease. However, further studies comprising more cases are needed to investigate the clinical significance of IGF-1 and IGFBP-3 in lung cancer.

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