The Diagnostic Role of Neopterin in Lung Cancer

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ABSTRACT

The aim of this study was to investigate the relationship between bronchoalveolar lavage (BAL) neopterin levels and lung cancer subtypes. Serum and BAL fluid samples were collected from lung cancer patients and control cases. BAL and serum neopterin levels were measured spectrofotometrically using the Neopterin ELISA kit. A total of 43 patients, 36 male and 7 female, who applied to our hospital were included in this cross sectional study. Thirty-two of the patients had confirmed primary lung cancer, while the 11 patients who formed the control group had benign lung diseases. The mean age in the lung cancer group was 59.8 ± 7.9, while for the control group it was 47.6 ± 8.2 years. The standardized BAL neopterin levels were 2.63 ± 1.53 nmol/L in NSCLC patients, 2.09 ± 1.02 nmol/L in SCLC patients, 2.36 ± 0.83 nmol/L in control groups. No significant difference was found. For patients with NSCLC, no correlation could be established between serum and BAL neopterin levels and stage of disease. In conclusion the standardized BAL and serum neopterin levels were inadequate to distinguish the lung cancer from the control group.

Keywords: Bronchoalveolar lavage, Lung cancer, Neopterin, Serum

ÖZET

Akciğer Kanserinde Neopterinin Tanısal Değeri

Çalışmanın amacı akciğer kanseri alt tipleriyle bronkoalveoler lavaj (BAL) neopterin düzeyleri arasındaki ilişkiyi araştırmaktır. Akciğer kanserli hastalardan ve kontrol vakalardan serum ve BAL sıvı örnekleri toplandı. BAL ve serum neopterin düzeyleri Neopterin ELISA kiti kullanılarak spektrofotometrik olarak ölçüldü. Hastanemize başvuran toplam 43 hasta bu kesitsel çalışmaya dahil edildi. Benign akciğer hastalıklarına sahip 11 hasta kontrol grubunu oluştururken, 32 hastada primer akciğer kanseri teyit edilmişti. Akciğer kanseri grubunda ortalama yaş 59.8±7.9, kontrol grubunda ise 47.6±8.2 yıl idi. Standartlaştırılan BAL neopterin düzeyi küçük hücreli dışı akciğer kanseri (KHDAK) hastalarında 2.63 ± 1.53 nmol/L, küçük hücreli akciğer kanseri (KHAK) hastalarında 2.09 ± 1.02 nmol/L, kontrol grubunda 2.36 ± 0.83 nmol/L idi. Gruplar arasında anlamlı fark saptanmadı. KHDAK'lı hastalar için, serum ve BAL neopterin düzeyleri ve hastalığın evresi arasında ilişki kurulamadı. Sonuçta, standartlaştırılan BAL ve serum neopterin düzeyleri, akciğer kanserini kontrol grubundan ayırmada yetersiz kalmaktadır.

Anahtar Kelimeler: Bronkoalveoler lavaj, Akciğer kanseri, Neopterin, Serum

INTRODUCTION

Lung cancer is the leading cause of cancer death among all cancers in both women and men.[1] The 5-year survival rate for non-small cell lung cancer (NSCLC) (all stages combined) is about 15%, in spite of improvements in therapy.[2] As for all malignant diseases, early diagnosis is key in successful management of lung cancer. Neopterin, a pteridine derivative, is produced by monocyte-derived macrophages upon stimulation with pro-inflammatory cytokine interferon-γ.[3] There are studies reporting that neopterin concentrations increase in malign diseases, particularly hematological, gynecological, gastrointestinal and lung cancers, in association with cellular immune response.[3-9] To date, only one study examined the role of bronchoalveolar lavage (BAL) neopterin in the lung cancer patients.[3] The aim of the present study is to investigate the diagnostic value of neopterin levels in serum and BAL, which mirror most accurately the characteristics of lung alveolar system, the primary site of involvement.

MATERIALS and METHODS

A total of 43 patients, 36 male and 7 female, who presented to Ataturk Chest Diseases and Thoracic Surgery Teaching and Research Hospital were included in this cross sectional study. Thirty-two of the patients had confirmed primary lung cancer, while 11 patients who formed the control group had benign lung diseases.

The histopathological diagnosis of primary lung cancer was made using bronchosopic forceps biopsy, transthoracic needle aspiration, thoracotomy, lymph node biopsy and pleural biopsy. For staging purposes, American Joint Committee on Cancer (AJCC) was used for NSCLC, and for small cell lung cancer (SCLC) the Veterans Administration Lung Study Group (VALG) staging system was preferred. All patients underwent routine physical and radiological examination, complete blood count, blood biochemistry workup, fiberoptic bronchoscopy (FOB), abdominal ultrasonography, computed tomography of the chest and head, and bone scintigraphy. Informed consent was obtained before every bronchoscopic procedure, and the study was undertaken with the approval of the local ethics committee.

BAL material was obtained using Olympus BF Type IT 20 D FOB at Ataturk Chest Diseases and Thoracic Surgery Teaching and Research Hospital. Patients with proximal endobronchial lesions resulting in total or near-total occlusion of the bronchi with hemorrhagic lavage, those who were positive for HIV, HBV and HCV serology, those with congestive heart failure, chronic liver disease, chronic renal failure, active lung tuberculosis, diabetes mellitus, non-pulmonary malignancies or any autoimmune disorder, as well as pregnant women were excluded from the study. BAL was obtained after the FOB was introduced as far into the affected segment as possible, followed by the injection of 100 ml of sterile serum physiologic in increments of 20 ml, which was then aspirated until at least 50-60% of the injected fluid was reacquired. Biopsies and brush cytology samples were obtained after BAL was collected. For the control group, BAL samples were obtained from the middle lobe or lingular segment.[3] Samples were sent to the Faculty of Pharmacy at Gulhane Military Medical Academy for the measurement of BAL neopterin levels.

Blood samples were obtained after FOB was performed, after which a 20 minute period was allowed for coagulation. Serum was obtained after the samples were centrifuged for 8 minutes at 4000 rpm. The serum and BAL samples were stored away from light at a temperature of -20^{0}C. BAL and serum neopterin levels were measured spectrofotometrically using the Neopterin ELISA kit (IBL, Hamburg-Germany). To standardize neopterin measurements from the BAL samples, the relative concentrations of urea, another molecule that crosses into BAL fluid by passive diffusion, were used as a reference.[1] Serum and BAL urea levels were measured using the sigma diagnostic BUN commercial kit, and the urea levels were calculated from the obtained values. Results were given as mean ± standard deviation. Comparison of groups was done using Mann-Whitney-U, Kruskal Wallis and Pearson tests where relevant (SPSS for windows). A p value of <0.05 was considered statistically significant.

RESULTS

A total of 43 patients were included in the final analysis, 32 of which had lung cancer while 11

Table 1. Patient charecteristics

	NSCLC		SCLC		Control group	
Number of patients	24		8		11	
Male/Female	21/3		7/1		8/3	
Mean Age (years)	59.1±9.1 (range, 42-75)		62.1±7.0 (range, 50-69)		47.6±8.2 (range, 31-60)	
Cigarette smoking	20 (%83.3)		7 (%87.5)		8 (%72.7)	
Diagnosis		**n**		**n**		**n**
	Epidermoid carcinoma	11	Limited	5	COPD	6
	Adenocarcinoma	9	Extensive	3	Bronchiectasis	2
	Other type	4			Delayed resolution of pneumonia	2
					Resolved Tuberculosis	1
Stage	IA / IB	2 / 3				
	IIA / IIB	- / 1				
	IIIA / IIIB	3 / 5				
	IV	10				

n= number of patients; NSCLC= non-small cell lung cancer; SCLC= small cell lung cancer; COPD= chronic obstructive pulmonary disease

patients who formed the control group were diagnosed with a benign lung disorder. Of the patients in the control group who were investigated for hemoptysis, 4 had chronic bronchitic changes while 2 had bronchiectasia. In two patients who were investigated for hilar fullness, this was attributed to pulmonary hypertension. Patient characteristics, diagnosis and their frequencies were summarized in Table 1. The mean age in the lung cancer group was 59.8 ± 7.9, while for the control group it was 47.6 ± 8.2 years. The age difference between the two groups was not statistically significant (p>0.05). The serum and BAL neopterin levels of three groups were shown in Table 2.

The highest serum neopterin levels were observed in adenocancer histological type in NSCLC group. When serum neopterin levels compared between control and lung cancer groups including subtypes, no significant difference was found (p>0.05). The difference between NSCLC and SCLC groups was not also statistically significant (p>0.05). No correlation could be established between serum neopterin levels and age. Serum neopterin levels did not differ between smoking and non-smoking subjects within NSLC patients (p>0.05). No significant difference in standardized BAL neopterin levels was found between control and lung cancer group including subtypes (p>0.05). For patients with NSCLC, no correlation could be established between serum and BAL neopterin levels and stage of disease. The number of patients with SCLC was insufficient to allow statistical analysis of relation between serum and BAL levels and different stages of cancer.

DISCUSSION

Lung cancer is the most common cause of cancer-related death in men and women in US.[1] As for all malignant diseases; early diagnosis is the key in the successful management of lung cancer. Early diagnosis, the proper assessment of extent of disease and the evaluation of treatment response require the use of biomarker assays in body fluids such as BAL, CSF, pleural fluid, blood.[10] Recently several studies on lung cancer have reported on the use of peptide hormones, enzymes, tumor tissue antigens, and genes as biochemical markers, none of which have been proven to be tumor specific and well es-

Table 2. Comparison of quantitative Neopterin levels in control group and in the patients with lung cancer and subtypes

Groups	n	Serum (nmol/L)	Standardized BAL (nmol/L)
Control	11	25.78 ± 12.07 (range 7.61 - 50.71)	2.36 ± 0.83 (range 0.38-3.27)
Lung Cancer	32	37.00 ± 22.71 (range 8.17 - 94.36)	2.49 ± 1.42 (range 0.20 - 6.06)
NSCLC	24	38.7 ± 21.31 (range 8.65 – 88.47)	2.63 ± 1.53 (range 0.20 - 6.06)
Stage I, II, IIIA		40.97 ± 25.9	2.74 ± 1.96
Stage IIIB-IV		37.33 ± 19.38	2.56 ± 1.28
SCLC	8	31.90 ± 27.43 (range 8.17 - 94.36)	2.09 ± 1.02 (range 0.33 - 3.22)

n= number of patients; NSCLC= non-small cell lung cancer; SCLC= small cell lung cancer; BAL= bronchoalveolar lavage

tablished molecular prognostic factor.[11] Mild elevations in the levels of tumor markers are known to occur in benign lung disorders, both in the tissue and in body fluids. The highest specificity reported for the many tumor markers studied does not exceed 70%.[12-15] For this reason, rather than being diagnostic, the use of tumor markers in patients with suspected malignancies has been relegated to a supportive role.

Neopterin is secreted by peripheral blood monocytes/macrophages stimulated by gamma-interferon which in itself is produced as a result of T lymphocyte activation. The detection of neopterin in different body fluids in vivo, have led to the investigation of its value as a tumor marker and a prognostic factor for malignancies. Earlier studies have demonstrated increases in neopterin levels in relation to tumor type and stage.[3-5] In studies on the diagnostic value of neopterin for hematological malignancies, high levels have been reported especially in patients with active leukemia and lymphoma, with substantially reduced levels in patients in remission.[6]

Similarly, studies on gynecological cancers such as ovarian, endometrial and vulvar cancer have demonstrated high serum and urinary levels of neopterin. More importantly, these studies established a correlation between clinical-radiological findings and neopterin levels during the follow-up period. Early stage urological malignancies were associated with lower levels of neopterin, which increased with progression of disease stage, with the exception of renal cell carcionoma.[7-9] Only a few studies have evaluated the diagnostic and prognostic value of neopterin for lung cancer.[3,16,17] Conrad et al.[16] reported increases in urinary neopterin levels in 58% of lung cancer patients, with extensive SCLC associated with higher levels compared to limited stage disease. Kronberger et al.[17] reported similar elevations in neopterin levels, however they also managed to demonstrate its value as a prognostic factor.

The diagnostic value of neopterin in BAL was investigated in 2001 by Mohamed et al.[3] Recently, investigators have suggested that the measurement of serum neopterin may not be ideal, and have advocated instead that measuring levels in more locally located fluids such as BAL may prove more beneficial as a tumor marker. BAL is a diluted material, and for standardization of neopterin levels the relative concentrations of serum and BAL levels of urea, which crosses into the BAL via passive diffusion, are used as a reference. In our study, BAL neopterin levels were standardized accordingly, and no significant difference between groups could be ascertained. The serum and BAL neopterin levels were found to be correlated in both the control and the lung cancer group, suggesting that the passage of neopterin into the BAL was unhindered.[3]

When patients with NCSLC where evaluated according to stage (I, II, IIIa, IIIb ve IV), the difference between stages with regard to mean serum and BAL neopterin levels was not statistically significant. In contrast, previous studies have actually established a link between neopterin levels and tumor load as well as stage of disease, with neopterin proving to be a significant prognostic marker.[3]

Prommeggar et al.[4] investigated the prognostic value of neopterin in 110 patients scheduled for lung cancer surgery. They found that preoperative neopterin levels were a valuable prognostic marker with regard to survival, and recommended that patients who were clinically operable should be reassessed before undertaking surgery if found to have high neopterin levels. It has been suggested that in patients with malignant disease high neopterin levels were associated with shorter survival.[3,4] The first such link in lung cancer patients was reported by Conrad et al.[16] in 1987, which was later consolidated by the findings of Kronberger et al.[17] In both studies, a decrease in survival periods was observed with every increase in neopterin levels.

In conclusion, neopterin levels have been shown to increase in many different malignant tumors, and its overexpression in the setting of tumor development and progression of disease makes it a potential indicator of neoplastic growth. However, in this study we failed to demonstrate any diagnostic value in lung cancer patients when compared to the control group. There remains a need for more extensive research on the diagnostic and prognostic value of neopterin.

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