The inflammatory cytokine interleukin-23 is elevated in lung cancer, particularly small cell type

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Aim of the study: Interleukin (IL)-17 and IL-23 play roles in inflammation and autoimmunity. The function of the IL-17/IL-23 pathway has not been completely evaluated in cancer patients. We aimed to investigate serum IL-17 and IL-23 levels and their relationship with clinicopathological and biochemical parameters in lung cancer patients.

Material and methods: Forty-five lung cancer patients and 46 healthy volunteers were included in the study. IL-17 and IL-23 measurements were made with the ELISA method. The ages of patients (53–84 years) and healthy subjects (42–82 years) were similar.

Results: Serum IL-23 levels were higher in lung cancer patients than in healthy subjects (491.27 ±1263.38 pg/ml vs. 240.51 ±233.18 pg/ml; $p = 0.032$). IL-23 values were higher in small cell lung cancer (SCLC) patients than in non-small cell lung cancer (NSCLC) patients (1325.30 ±2478.06 pg/ml vs. 229.15 ±103.22 pg/ml; $p = 0.043$). Serum IL-17 levels were lower in the patients, but the difference was not statistically significant (135.94 ±52.36 pg/ml vs. 171.33 ±133.51 pg/ml; $p = 0.124$). Presence of comorbid disease (diabetes mellitus, hypertension or chronic obstructive lung disease) did not have any effect on the levels of IL-17 or IL-23. Erythrocyte sedimentation rate values were positively correlated with cytokine levels, but serum albumin levels were negatively correlated.

Conclusions: Serum IL-23 levels are elevated in lung cancer patients, particularly those with SCLC. IL-17 and IL-23 values are correlated with inflammatory markers in the patients.

Key words: IL-17, IL-23, lung cancer, Th17 cells.

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Introduction

Lung cancer is one of the most common cancers in the world. It has the highest mortality rate. According to the etiological factors such as genetic modification, treatment has been individualized in lung cancer [1]. Thus explanation of etiologic factors is important. Besides smoking, many genetic and environmental factors also play a role in the etiology of lung cancer.

Inflammation is associated with several malignancies. It may be related to the initiation, promotion, progression, surveillance, and control of the malignant process [2]. Chronic inflammation may create structural, genetic, and epigenetic alterations in tumor initiation. Moreover, tumor-associated inflammation promotes tumor growth and metastatic formation. Inflammatory markers such as C-reactive protein (CRP) and high-density lipoprotein cholesterol (HDL cholesterol) have been altered and have prognostic value in lung cancer patients [3, 4].

CD4+ T helper (Th) cells are mediators of the cellular immune response. Th cells were first referred to as Th1 and Th2 due to cytokine expression patterns. Then Th17 cells, a subset of CD4+ T cells, were described. These cells produce IL-17, IL-21, and IL-22 and play a role in inflammation and autoimmunity [5]. IL-17 secretion is regulated by several factors, such as IL-23. IL-23 is secreted in dendritic cells and mononuclear macrophages [6]. These inflammatory cytokines, IL-23 and IL-17, were found to play important pathogenic roles in several inflammation related carcinomas. IL-23 and IL-23 receptors have been found elevated and IL-17A has been linked to an adverse prognostic outcome and rapid progression to metastatic disease in colorectal carcinomas. In animal models of colorectal carcinogenesis inhibition of these cytokines attenuates tumor development and malignant progression [6].

Although IL-17 and IL-23 play roles in several biological processes, the function of the IL-17/IL-23 pathway has not been completely evaluated in lung cancer patients. We investigated serum IL-17 and IL-23 levels and their relationship with clinicopathological and biochemical parameters in lung cancer patients.

Material and methods

Forty-six lung cancer patients and 45 control subjects were included in the study. The patients were treated in a medical oncology department; the healthy subjects were referred from check-up and chest medicine clinics and did not have any pathologic condition except chronic obstructive pulmonary disease (COPD), hypertension (HT), or diabetes mellitus (DM). Written informed consent was obtained from all participants. This study was approved

by the local ethical committee in December 2012. Demographic and clinical data were collected from clinical documentation.

Patients with chronic inflammatory diseases (except COPD, HT, or DM), rheumatic diseases, or primary immune deficiency, and those who used immunosuppressive medications were not included in the study.

Enzyme-linked immunosorbant assay (ELISA) and biochemical analyses

All biochemical investigations were performed under the same conditions in the Laboratory of Biochemistry, GATA Haydarpasa Hospital. The serum samples were centrifuged at 5000 RPM for 5 minutes. The supernatants were divided and collected at –80°C until the test day. The following parameters were determined:

- IL-17 was determined using Human IL-17 ELISA Kit (BOSTER Immunoleader No: EK0430, Boster Biological Technology Co., Ltd, 40459, Fremont, CA 94538, USA). Boster's human IL-17 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The measurement range of the assay is 31.2–2000 pg/ml and sensitivity is < 1 pg/ml;
- IL-23 was determined using the Human IL-23 ELISA Kit (OmniKine No: OK-0243, Assay Biotechnology Company, CA 94089, USA). The measurement range of the assay is 63-8000 pg/ml and sensitivity is < 25 pg/ml.

The measurements were performed according to the manufacturer's protocol. At the end readings were made directly at 450 nm with a microplate reader (DAR800, Diagnostic Automation, CA 91302, USA). The results were expressed as pg/ml.

Statistics

SPSS software was used for statistical analysis. All data are given as mean ± standard error of the mean (SEM). Normal distributions of group parameters were described using the Kolmogorov-Smirnov test. The Mann-Whitney rank sum test was used to compare differences between groups. Fisher's exact was used for categorical variables. Correlation between groups was calculated using Pearson's correlation test. Statistical significance was accepted if $p < 0.05$.

Results

Forty-six lung cancer patients and 45 controls were included in the study. Among patients, 35 had non-small cell lung cancer (NSCLC) (18 squamous cell carcinoma, 15 adenocarcinoma, 1 adenosquamous carcinoma, and 1 NSCLC, not otherwise specified), and 11 had small cell lung cancer (SCLC). Eight SCLC patients had extensive stage, and 3 had limited disease. Fourteen NSCLC patients were metastatic (11 stage III, 5 stage II, and 5 stage I). Lung cancer group and control groups were not different with regard to age, sex, smoking status, or comorbidities (Table 1).

In biochemical parameters, acute-phase reactants (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], ferritin, and serum albumin level) were different in lung cancer patients than in controls (data not shown). Leukocyte count, platelet count, neutrophil count, monocyte count, plateletcrit, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transpeptidase (GGT) were elevated; creatinine kinase, HDL cholesterol, serum sodium level, hemoglobin, and hematocrit were lower in lung cancer patients.

Serum IL-17 levels were lower in lung cancer patients than in controls, but the difference was not statistically significant. However, serum IL-23 level was higher in patients than in controls (Table 2). Among lung cancer patients, serum IL-23 levels of SCLC patients were higher than in NSCLC patients. Serum IL-17 levels were similar in both NSCLC and SCLC patients (Table 2). In lung cancer

Table 1. Demographics of lung cancer patients and controls

Parameter	Lung cancer patients		Control subjects		*p*
	n	%	*n*	%	
Sex					
Male	41	89.1	35	77.78	0.17
Female	5	10.9	10	22.22	
Comorbidity					
COPD	8	17.4	12	26.7	0.32
Hypertension	21	45.7	20	44.4	1.00
Diabetes mellitus	9	19.6	3	6.7	0.12
Smoking					
Smoker	27	58.7	21	46.7	0.08
Non-smoker	10	21.7	18	40	
Ex-smoker	9	19.6	6	13.3	
Ages (range)	(42–82)		(53–84)		0.10

COPD – chronic obstructive pulmonary disease

Table 2. Serum IL-17 and IL-23 levels in lung cancer patients and controls

Parameter	IL-17		IL-23	
		*p**		*p**
Groups				
Lung cancer patients	135.94 ±52.36		491.27 ±1263.38	
Control subjets	171.33 ±133.51	0.124	240.51 ±233.18	0.032
Lung cancer patients				
SCLC	144.89 ±57.81		1325.3 ±2478.06	
NSCLC	133.13 ±51.11	0.542	229.15 ±103.22	0.043
NSCLC				
Non-metastatic	136.84 ±50.38		246.34 ±98.13	
Metastatic	127.57 ±53.59	0.727	203.37 ±108.88	0,235

SCLC – small cell lung cancer; NSCLC – non-small cell lung cancer
**Mann-Whitney U test*

patients, serum IL-17 levels were not different between hypertensive and normotensive, diabetic and non-diabetic, or COPD and non-COPD patients. IL-23 levels were lower in diabetic and COPD patients than in non-diabetic and non-COPD patients, but the difference was not statistically significant in this small subpopulation of the study (Table 3). Levels of these interleukins were similar in metastatic and non-metastatic NSCLC patients and not different among patients of different sexes (Table 2).

ESR had a positive correlation with IL-17 and IL-23 levels (respectively r = +0.525; p = 0.002 and r: +0.399; p = 0.021). A negative correlation was detected between serum albumin and IL-17 level (r = –0.418/p = 0.004), but not IL-23. High serum creatinine level was associated with high serum IL-23 level (r = +0.294/p = 0.047) (not IL-17). Age, serum ferritin level, CRP or other biochemical parameters did not correlate with serum interleukin levels.

Discussion

In this study, we investigated two inflammatory cytokines, IL-17 and IL-23, in peripheral blood from lung cancer patients. We found that serum IL-23 level is elevated in lung cancer, particularly SCLC patients, but not IL-17. Both interleukins correlate with some inflammatory markers (e.g. ESR, albumin). This study has some limitations. First, the study population was small, so subgroup or survival analyses could not be performed. Second, Th17 cells and regulatory T (Treg) cell counts were not studied. To the best of our knowledge, this study is the first to investigate serum IL-17 and IL-23 levels together in lung cancer patients.

The relationship between IL-17 and cancer has already been established [7]. In the initial stage of tumorigenesis, increasing IL-17 presence has been demonstrated in the tumor microenvironment [8]. IL-17 is released as a response to the secretion of lactate via IL-23 dependent and independent pathways [9]. The increase in IL-17 can trigger the release of IL-6 and activation of the STAT3 pathway and NF-κB [10, 11]. Beside the tumor microenvironment, IL-17 has also been found at a high level in malignant pleural effusion [12, 13].

There are also studies about whether IL-17 level is a serum marker in cancer patients. In lung cancer patients, some studies have reported that serum IL-17 is high and a prognostic marker compared to healthy subjects [14, 15], but some showed that it is not different [15]. Xu *et al*. [14] investigated the baseline serum level of IL-17 in NSCLC patients. They found that serum IL-17 level is higher in patients than healthy subjects and high serum IL-17 concentration correlates with shorter survival and presence of metastasis. Li *et al*. [15] also observed an increased IL-17 level and increased TH17 cell and Treg cell counts in NSCLC patients. However, Brusino *et al*. [16] reported that serum IL-17 level was similar in lung cancer patients as in healthy volunteers, although its level was higher in exhaled breath condensate. In addition, Teng *et al*. stated that despite the clear link between IL-23 and Th17 cells, the role of IL-17 in tumor development has not been fully explained [17]. In our present study, we were unable to show that serum IL-17 level is different in lung cancer patients, although we found increasing Treg cells in a previous study [18] and immunosuppressive HLA-G expression (gastric cancer) [19].

Interleukin 23, another study cytokine, supports Th17 cell propagation, whose development is induced by IL-6 and TGF-β [20]. IL-17 plays a role together with IL-23 in several biologic processes; thus it is named the IL-23/IL-17 pathway. Among these biologic processes, the relationship of cancer with IL-23, like IL-17, has been investigated in previous studies. Interleukin 23 is also elevated in the tumor microenvironment [21, 22] and is associated with poor prognosis [23]. It was also found that IL-23 was elevated in serum from cancer patients. Ljujic *et al*. [24] observed a higher serum IL-23 level and its correlation with tissue expression of vascular endothelial growth factor (VEGF) in colorectal cancer (CRC) patients. Adamo *et al*. [25] reported an increased serum IL-23 level in resected CRC and post-chemotherapy CRC patients compared to healthy subjects. Stanilov *et al*. [26] investigated the association

Table 3. Serum IL-17 and IL-23 levels and comorbidities in lung cancer patients

Parameter	IL-17		IL-23	
		p		*p*
Hypertension				
Hypertensive	143.04 ±38.36		424.76 ±853.3	
Normotensive	129.98 ±61.92	0.201	547.14 ±1542.36	0.877
Diabetes Mellitus				
Diabetic	131.66 ±55.75		267.11 ±134.08	
Non-diabetic	136.98 ±52.26	0.935	545.8 ±1405.55	0.765
COPD				
COPD patients	110.87 ±39.19		248,14 ±106.37	
Non-COPD	141.22 ±53.66	0.201	542,46 ±1386.95	0.898

** Mann-Whitney U test*

of serum levels of IL-23 and IL-12p40 with survival of patients with CRC. Interleukin 23 was not a prognostic marker. Gangemi *et al.* [27] found that IL-23 level was higher in breast cancer patients than controls. They claimed that a higher IL-23 value has negative prognostic significance. Besides these cancer studies, IL-23 levels have not been investigated in serum from lung cancer patients. In our study, we have shown for the first time that serum IL-23 is elevated in lung cancer patients compared to healthy subjects. Interleukin 23 level is increased more in SCLC than NSCLC patients.

In conclusion, serum IL-23 level is elevated in lung cancer, particularly SCLC patients, but not IL-17. While the prognostic value of the IL-17/IL-23 pathway was not addressed in this study, further studies including more cases are required.

The authors declare no conflict of interest.

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