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NEÜ YAYINEVİ

The Relationship Between Neutrophil Elastase, Il-1β, Il-8 and Desmosin Levels in Sputum and Blood with Sputum Culture Results in Bronchiectasia Patients

Bronşektazili Hastalarda Balgam ve Kandaki Nötrofil Elastaz, Il-1β, Il-8 ve Desmosin Düzeylerinin Balgam Kültürü Sonuçlari İle İlişkisi

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Öz

Amaç: Çalışmamızda, bu hastalarda kan ve balgamda NE, kanda IL-8, IL-1β ve desmosin düzeylerinin tanısal değerini ve semptomlarla, alevlenme sıklığıyla, etiyolojileriyle, radyolojik tutulum yaygınlığıyla, bronşektazi tipleriyle ilişkilerini araştırmayı amaçladık.
Hastalar ve Yöntem: Çalışmaya Kasım 2020-Mart 2021 tarihleri arasında Göğüs hastalıkları polikliniğine başvuran ve kliniğimizce takipli 18-90 yaş arası 46 bronşektazili hasta ve 45 kişilik kontrol grubu alındı.
Bulgular: Kanda NE, IL-1β, desmosin hasta grubunda istatistiksel olarak anlamlı derecede yüksekti ($p<0,001$). Biyobelirteçlerin tanısal değeri için ROC analizi yapıldı. Kan NE için kesme değeri 12,70 ng/mL olduğunda duyarlılık %71,7, özgüllük %77,8, IL 1β için kesme değeri 2,935 pg/mL, duyarlılık %73,9, özgüllük ise %73,9 olarak belirlendi. %71,1, desmozin için eşik değeri 0,505 ng/mL, duyarlılığı %67,4 ve özgüllüğü %62,2 idi.
Sonuç: Çalışmamızda bronşektazili hastalarda kanda NE, IL-1β ve desmosinin ve balgamda NE'nin önemli inflamatuar belirteçler olduğu, önemli tanısal değere sahip olduğu ve takip parametrelerinin önemli bir bileşeni olabileceği belirlendi. bu parametreler alevlenmelerin sıklığı ile önemli ölçüde ilişkilidir.

Anahtar Kelimeler: Bronşektazi, nötrofil elastaz, desmozin, IL-8, IL-1β, balgam kültürü

Abstract

Aim: In our study, we aimed to investigate the diagnostic value of NE in blood and sputum, blood IL-8, IL-1β and desmosine levels and their relationship with symptoms, frequency of exacerbation, etiology, prevalence of radiological involvement, types of bronchiectasis, as well as sputum cell counts and cultures.
Patients and Methods: The study included 46 patients with bronchiectasis aged between 18-90 years, who applied to the Chest Diseases Polyclinic between November 2020 and March 2021 and were followed up by our clinic, and a control group of 45 people.
Results: At blood, NE, IL-1β, desmosine were statistically significantly higher in the patient group ($p<0.001$). ROC analysis was performed for the diagnostic value of biomarkers. When the cut-off value for blood NE was 12.70 ng/mL, the sensitivity was 71.7%, the specificity was 77.8%, for IL 1β the cut-off value was 2.935 pg/mL, the sensitivity was 73.9%, the specificity was 71.1%, for desmosine, the cut-off value was 0.505 ng/mL, the sensitivity was 67.4% and the specificity was 62.2%.
Conclusion: In our study, it was found that NE, IL-1β and desmosine in blood and NE in sputum in patients with bronchiectasis are important inflammatory markers, have significant diagnostic value, and may be an important component of follow-up parameters, since these parameters are significantly correlated with the frequency of exacerbations.

Keywords: Bronchiectasis, neutrophil elastase, desmosine, IL-8, IL-1β, sputum culture

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INTRODUCTION

Bronchiectasis is a disease characterized by persistent bronchial dilatation associated with chronic neutrophilic airway inflammation (1). It usually presents with symptoms such as cough, sputum production, hemoptysis, recurrent lung infections, weakness and weight loss. In terms of pathophysiology, bronchial dilation leads to impaired mucociliary clearance. Failure to adequately clear bacteria and mucus from the airways causes permanent infection, inflammation, and airway damage. Progressive airway damage can lead to lung dysfunction, worsening of symptoms, and eventually respiratory failure and death. Bronchiectasis may be the last common pathway of severe infections, inflammatory, allergic, genetic and degenerative disorder. Thus, it is the result of multiple pathophysiological processes and one of the most complex and heterogeneous syndromes in respiratory medicine (2). Airway infection as well as exacerbations are critical events in bronchiectasis as they are associated with a marked reduction in quality of life and contribute to disease progression (3). Therefore, the majority of bronchiectasis treatment recommended by the European Respiratory Society (ERS) guidelines is aimed at suppressing airway infection or reducing the frequency of exacerbations (4,5). Biomarkers that can identify patients at risk of exacerbation or active airway infections in real time will help identify patients who need intensive treatment (6). There are limited number of studies on this subject.

Neutrophil elastase (NE), a serine protease produced by neutrophils and released in response to infectious stimuli, has been found to increase in amount during exacerbations of airway disease and decrease with treatment of the exacerbation and resolution of symptoms (7). Desmosin, a biomarker released during NE-related elastin degradation, also shows similar correlations (8). Tsang et al. showed a correlation between sputum neutrophil elastase, radiological involvement, and functional markers in a sample of 30 bronchiectasis patients (9). It was hypothesized that local or systemic antibiotic therapy and a consequent reduction in bacterial load reduce sputum NE levels. Previous findings suggest that short oral antibiotic therapy is effective in reducing NE activity in sputum (10-12). IL-8 is one of the most potent chemoattractants that degranulate neutrophils in the bronchiectatic airways. Long-term lung expression of IL-8 contributes to the double-edged sword of the inflammatory response in a variety of lung diseases, including chronic obstructive pulmonary disease (COPD), bronchiectasis, and cystic fibrosis. Lung-targeted IL-8 provides enhanced protection against bacterial infection in the lung and leads to changes in inflammation, mucus hypersecretion, pulmonary remodeling and fibrosis, damaged and decreased lung function. Thus, IL-8-mediated enhanced microbial immunity presents with a high level of progressive lung injury and reduced lung integrity (13). It has also been suggested that IL-8 is a pro-inflammatory marker in bronchiectasis, COPD and allergic asthma IL-1β mediates airway inflammation and fibrosis (14,15). Recently, it was shown that transient expression of IL-1β using an adenoviral vector can lead to progressive fibrosis long after IL-1β levels fall and the acute inflammatory response has ended (16). Inhibition of IL-1β at the onset of animal fibrosis models has been reported to result in disease regression (17). Elastin degradation as a result of NE activity results in the presence of elastin-derived peptides, including desmosine and isodesmosine, in serum, plasma, and urine (18). Increased circulating desmosine in patients with bronchiectasis was also associated with a higher risk of severe exacerbations. Since few clinical parameters have been shown to be associated with bronchiectasis, sputum neutrophil elastase and circulating desmosine may be helpful in clinical trials or in patient evaluation (19). In another recent analysis in which 3 large studies evaluated bronchiectasis (TAYBRIDGE), COPD (ECLIPSE study), abdominal aortic aneurysm (MA3RS trial), high circulating plasma desmosine level has been associated with increased mortality in all causes of mortality independent of the underlying disease process and has been suggested to be a useful universal prognostic biomarker in populations at risk (20).

In this study, in patients diagnosed with bronchiectasis clinically and radiologically (with high resolution computed tomography), we determined NE activity in blood and sputum, diagnostic value of blood desmosine and proinflammatory biomarker (IL-8, IL-1β) levels, correlations with symptoms, radiological extent, type of bronchiectasis, frequency of exacerbation, as well as sputum cell count and culture and aimed to investigate whether these biomarkers can be reliable markers in the diagnosis and follow-up of bronchiectasis.

PATIENTS AND METHODS

The study consisted of 46 patients with

bronchiectasis, aged 18-90 years, whose last 5-year follow-ups (PA chest X-ray, HRCT) could be accessed, and as a control group, who were followed up by our Medical Faculty Hospital Chest Diseases Clinic and admitted between november 2020 and march 2021, and 45 people who applied to the chest diseases outpatient clinic for reasons other than bronchiectasis, without any cardiopulmonary, metabolic, cancer, acute or chronic inflammatory disease. This study was conducted in accordance with the Declaration of Helsinki, and the study approval was obtained from the Non-Invasive Clinical Research Ethics Committee of Medical Faculty (Decision No: 2019/2083). Written and informed consent statements were obtained from all participants. The patients' exacerbation frequency, cough, sputum production, shortness of breath, chest pain and hemoptysis histories were questioned. By measuring the NE level in the sputum of the patients, NE, desmosine, IL-8 and IL-1beta levels in the serum of the patients, these biomarkers were measured for the duration of the disease, symptoms, extent of the disease (radiologically involved lobes and segments), bronchiectasis type, exacerbation frequency, frequency of hospitalization, smoking history and sputum cell count and their relationship with bacteria grown in culture, and the correlation of biomarkers among themselves were investigated. NE, desmosin, IL-8, IL-1beta levels in the blood were studied. Blood and sputum samples were taken from the patients upon admission to the hospital, before the start of treatment. Not all sampled patients were receiving oral corticosteroids or any antibiotic therapy.

In order to investigate the correlation of the biomarkers to be studied with the radiological extent of the disease and the frequency of exacerbations, the routine laboratory tests (hemogram and CRP), thorax CT and/or high resolution lung tomography (HRCT) of the patients in their 5-year retrospective follow-up were examined, and their reports were scanned and recorded from the hospital information system. Disease duration, smoking history, symptoms, frequency of exacerbations and hospitalizations, and additional diseases were recorded by questioning the patients. Sputum NE, serum NE, desmosin, IL-8 and IL-1 were analyzed by enzyme linked immunosorbent assay (ELISA) method in the study. When gram stained preparations made from sputum samples were examined under the microscope, samples containing less than 10 epithelial cells and more than 25 leukocytes in each area at 100 magnification were accepted as suitable sputum samples. Eosin methylene blue (EMB), chocolate agar and sheep blood agar medium were used for culture study of sputum samples. Identification of microorganisms thought to be causative was done by conventional methods, with automated systems when necessary.

The blood samples were centrifuged at 4 ºC, 1,000 g for 10 minutes in a Hettich Rotina 46R (Hettich Zentrifugen, Tuttlingen, Germany) refrigerated centrifuge device, and serum samples were separated. Serum samples were stored at -80 °C in a New Brunswick U570 (New Brunswick Scientific, New Jersey, USA) refrigerator until the levels of IL-1 beta, IL-8, desmosine and neutrophil elastase in the serum were studied. Sputum samples were stored in a New Brunswick U570 (New Brunswick Scientific, New Jersey, USA) refrigerator at -80 °C until NE levels in sputum were studied. BT-Lab (E0890Hu, Bioassay Technology Laboratory Inc., Shanghai, China) kit was used for measurement of serum neutrophil elastase level. Neutrophil elastase results were calculated as “ng/mL” according to absorbance-concentration calibration charts using the Bio-rad microplate absorbance reader xMark (Bio-rad Laboratories, California, USA) system. The sputum sample was weighed and vortexed by adding four solids (w/v) 0.1% dithiothreitol. Four times (w/v) of the weighed sputum sample was vortexed by adding 0.9% NaCl (Sputum/DTT/SF ratio was 1/4/4 as w/v/v, dilution factor was 9). The mixture was centrifuged in a Hettich Rotina 46R instrument at 4 ºC at 10,000 g for 10 minutes. Suprenatant was used for the measurement of neutrophil elastase level.[21, 22] BT-Lab (E0890Hu, Bioassay Technology Laboratory Inc., Shanghai, China) kit was used for measurement of sputum neutrophil elastase level Neutrophil elastase results were calculated as “ng/mL” according to absorbance-concentration calibration charts using the Bio-rad microplate absorbance reader xMark (Bio-rad Laboratories, California, USA) system. AndyGene (AD10775Hu, AndyGene Biotechnology, Beijing, China) kit was used to measure serum IL-1β level. IL-1 beta results were calculated as “pg/mL” according to absorbance-concentration calibration charts using the Bio-rad microplate absorbance reader xMark (Bio-rad Laboratories, California, USA) system. For measurement of serum IL-8 level, USCN (SEA080Hu, USCN Life Science. Inc., Wuhan, Çin)) kit was used. IL-8 results were calculated as “pg/mL” according to the absorbance-concentration calibration charts using the Bio-rad microplate absorbance reader xMark (Bio-rad Laboratories, California,

USA) system. The MyBioSource (MBS771228, MyBioSource Inc, California, USA) kit was used for measurement of serum desmosine level. Desmosine results were calculated as “ng/mL” according to the absorbance-concentration calibration charts using the Bio-rad microplate absorbance reader xMark (Bio-rad Laboratories, California, USA) system. Pregnant women, patients who did not accept to participate in the study, and patients with acute or chronic infection or inflammation, except for ronsiectasis, were excluded from the study.

Statistical Analysis:

The results of our study were analyzed with the SPSS (The Package for Social Sciences) 19.0 program. The conformity of the data to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogrorov-Smirnov/Shapiro-Wilk tests). In the evaluation of numerical data, arithmetic mean, standard deviation, median, minimum and maximum values were used and frequency distributions and percentages were used to summarize categorical data. Chi-square ($\chi 2$) test was used to compare categorical data. The relationship between normally distributed numerical data and categorical data was evaluated with the T test in independent groups, and the relationship between non-normally distributed numerical data and categorical data was evaluated with the Man-Whitney U test. Correlations of non-normally distributed numerical variables were analyzed with the Spearman correlation coefficient. Diagnostic decision-making properties of IL-1β, IL 8, Desmosine and blood neutrophil/elastase levels in predicting the disease were analyzed by ROC (Receiver Operating Characteristics) curve analysis. In the presence of significant breakpoints, the sensitivity, specificity, positive predictive value and negative predictive values of these limits were calculated. Type-1 error level was accepted as 5% for statistical significance. In the evaluation of Spearman correlation coefficients, 0.05-0.30 was considered a low or insignificant relationship, 0.30-0.40 a low-moderate relationship, 0.40-0.60 a moderate relationship, 0.60-0.70 a good relationship, a 0.70-0.75 a very good relationship, and 0.75-1.00 an excellent relationship. Correlation coefficients with positive signs indicate that the variables increase and decrease together, while correlation coefficients with negative signs indicate that while one of the variables increases, the other decreases or vice versa (23). We calculated a sample size of 27 in each group with an alpha error of 5% and power of 95.

RESULTS

In our study, out of 46 bronchiectasis patients, 19 (41.3%) were female, 27 (58.7%) were male, and in the control group, 18 (40%) of 45 patients were female and 27 (60%) were male. The mean age of the patients with bronchiectasis was 50.56±15.31 years. Active symptoms were present in 44 (95.7%) patients with bronchiectasis. Dyspnea and cough were present in 31 (67.4%), expectoration in 37 (80.4%) and hemoptysis in 2 (4.3%). Cystic bronchiectasis was found in 23 (50%) of the bronchiectasis patients, tubular bronchiectasis in 22 (47.8%), and varicose bronchiectasis in 1 (2.2%). 37 (80.4%) of 46 patients were able to produce sputum. In the microscopic examination of sputum, neutrophil dominance was observed in all samples. There was growth in culture in 9 (24.3%). P. aeruginosa was grown in 4 patients (44.4%), enterobactericea in 3 (33.3%), acinetobacter baumannii in 1 (11.1%) and klebsiella in 1 (11.1%). When their etiology was examined, eiology of unknown (idiopathic) was 21 (45.7%), childhood lower respiratory tract infection was 14 (30.4%), previous pneumonia was 6 (13%) childhood measles infection was 3 (6.5%), and previous tuberculosis was 2 (4.3%). Among the studied biomarkers, IL-1β (4.82±3.09 - 2.03±1.39, $p<0.001$), desmosine (0.71±0.54 - 0.45±0.12, $p<0.001$), blood neutrophil elastase (17.93±8.11-10.29±2.98, $p<0.001$), CRP (21.53±43.25 - 2.88±4.20, $p=0.003$) and blood neutrophil count (5.59±2.52 - 4.47±1.71, $p=0.026$) were statistically significantly higher in the bronchiectasis patient group. Sputum NE value in the patient group was 31.68±17.00 ng/mL (Table 1).

There was no statistically significant difference in IL1-β, IL-8, desmosine, blood NE, sputum NE levels according to the etiology of bronchiectasis patients. The data are shown in (Table 2).

In order to determine whether IL-1β, IL-8, desmosine, blood NE, sputum NE values changed according to the etiology of bronchiectasis, a comparison was made between them; no significant difference was found.

It was investigated whether there was a difference in IL-1β, IL-8, desmosine, blood NE, sputum neutrophil elastase values in patients with bronchiectasis between those with and without symptoms. No significant statistical difference was found.

When the IL-1β, IL-8, desmosine, blood NE and sputum NE levels of the patients were compared

Table 1. Comparison of biomarkers, CRP and hematological parameters between patients with bronchiectasis and control groups

	Patients with Bronchiectasis n:46		Control Group n:45		p
	Mean± SD	Min-max	Mean±SD	Min-max	
IL-1β (pg/mL)	4.82±3.09	1.94-14.86	2.03±1.39	0.57-5.36	0.000*
IL-8 (pg/mL)	173.58±187.13	8.80-662.5	116.95±101.44	13.50-289.20	0.284*
Desmosine (ng/mL)	0.71±0.54	0.33-3.29	0.45±0.12	0.18-0.67	0.000*
Blood NE (ng/mL)	17.93±8.11	5.60-34.40	10.29±2.98	4.90-15.90	0.000**
CRP	21.53±43.25	0.00-235.0	2.88±4.20	0.00-23.00	0.003*
WBC	8.39±2.87	3.14-16.82	7.95±2.29	4.76-14.01	0.531*
Blood Neutrophil	5.59±2.52	2.25-12.87	4.47±1.71	2.43-10.30	0.026*
Blood Lymphocyte	1.99±1.04	0.38-5.03	2.76±0.93	0.88-4.56	0.000*
Sputum NE (ng/mL) (n:37)	31.68±17.00	10.46-73.68			

IL-1β: Interleukin 1beta, IL-8: Interleukin-8, NE: Neutrophil elastase, n: Number of patients, *=Mann Whitney-U test, **=independet samples t-test

Table 2. Correlation of CRP, WBC, neutrophil, lymphocyte counts, radiological involvement grades, exacerbation frequency, number of hospitalizations and smoking history of patients with bronchiectasis with biomarkers.

	IL-1β	IL 8	Desmosine	Blood NE	CRP	WBC	Blood Neutrophil	Blood Lymphocyte	Affected Lobe	Exacerbation	Hospitalization	Smoking History	Sputum NE
Disease period	0.188	-0.172	0.225	0.253	0.252	0.244	0.204	0.048	**0.324***	**0.770****	**0.399****	-0.145	0.146
p	0.212	0.253	0.133	0.090	0.091	0.102	0.173	0.751	**0.028**	**0.000**	**0.006**	0.543	0.387
IL-1β		0.033	**0.964****	**0.899****	-0.071	-0.040	-0.074	-0.033	0.201	**0.310***	0.050	-0.072	**0.876****
p		0.827	**0.000**	**0.000**	0.640	0.790	0.626	0.828	0.181	**0.036**	0.739	0.763	**0.000**
IL 8			-0.053	-0.061	-0.059	0.074	-0.004	0.079	-0.098	-0.021	-0.091	-0.079	0.042
p			0.727	0.687	0.696	0.626	0.977	0.603	0.516	0.890	0.548	0.739	0.807
Desmosine				**0.944****	-0.113	-0.057	-0.083	-0.046	0.244	**0.328***	0.096	-0.030	**0.922****
p				**0.000**	0.453	0.705	0.583	0.764	0.102	**0.026**	0.525	0.900	**0.000**
Blood NE					-0.224	-0.047	-0.091	0.000	0.146	**0.316***	0.032	0.088	**0.982****
p					0.135	0.756	0.547	0.998	0.334	**0.032**	0.835	0.711	**0.000**
CRP						**0.394****	**0.556****	**-0.433****	0.277	**0.324***	**0.447****	-0.094	-0.275
p						**0.007**	**0.000**	**0.003**	0.062	**0.028**	**0.002**	0.693	0.100
WBC							**0.895****	0.273	0.196	**0.332***	0.207	0.361	-0.002
p							**0.000**	0.067	0.192	**0.024**	0.167	0.118	0.989
Blood Neutrophil								-0.090	0.252	0.287	0.242	**0.452***	-0.084
p								0.551	0.091	0.053	0.105	**0.045**	0.620
Blood Lymphocyte									-0.102	0.014	-0.191	-0.098	0.155
p									0.502	0.926	0.204	0.680	0.360
Affected Lobe										**0.509****	**0.547****	-0.010	0.048
p										**0.000**	**0.000**	0.965	0.777
Exacerbation me											**0.692****	0.063	0.246
p											**0.000**	0.791	0.143
Hospitalization												0.067	-0.044
p												0.780	0.796
Smoking history													-0.133
p													0.636

IL-1β: Interleukin 1beta, IL-8: Interleukin-8, NE: Neutrophil elastase, * p <0.05, **p <0.01

Table 3. ROC analysis parameters of IL-1β, IL-8, desmosine and blood NE in patients with bronchiectasis

Biomarkers	AUC (Area under the curve)	95% Confidence Interval	p
IL 1β	0.832	0.747-0.916	0.000
Desmosine	0.717	0.514-0.821	0.000
Blood NE	0.786	0.688-0.884	0.000
IL 8	0.565	0.446-0.685	0.284

Table 4. Cut-off values, sensitivity, specificity, PPD and NPDs for IL-1β, desmosine and blood NE

Biomarkers	Cut-off value	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
IL-1β	2.935 pg/mL	73.9	71.1	72.3	72.7
Blood NE	12.70 ng/mL	71.7	77.8	76.7	72.9
Desmosine	0.505 ng/mL	67.4	62.2	64.6	65.1

IL-1β:Interleukin 1β, NE: Neutrophil elastase

according to the bronchiectasis type, no statistically significant difference was found between them.ROC analysis was performed to determine the diagnostic value, sensitivity, specificity, positive and negative predictive values of IL-1β, IL-8, desmosine, and blood NE (Table 3).

The cut-off value was not calculated for IL-8, since the AUC (Area under the curve) value was found to be insignificant (Figure 1). When the cut-off value for IL-1β was taken as 2.935 pg/mL in the diagnosis of bronchiectasis, its sensitivity was 73.9%, specificity was 71.1%, positive predictive value (PPD) was 72.3%, and negative predictive value (NPD) was 72.7%. When the cut-off value for desmosine was 0.505 ng/mL, the sensitivity was 67.4%, specificity was 62.2%, PPD was 64.6%, NPD was 65.1%. When the cut-off value for blood NE was taken as 12.70 ng/mL, the sensitivity was 71.7%, the specificity was 77.8%, PPD was 76.7%, and NPV was 72.9% (Table 4). No significant cut-off value with high sensitivity and specificity was found for IL-8 level, one of the parameters studied.

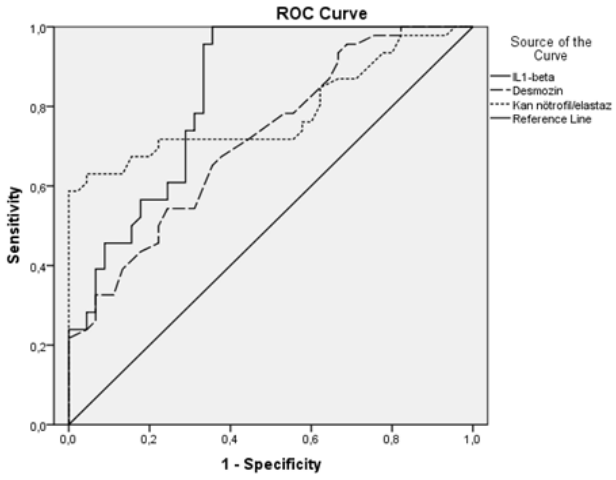

Figure 1. ROC curves for IL-1β, desmosine, and blood NE

DISCUSSION

Neutrophils play a key role in the development and progression of bronchiectasis (24). Patients with clinically stable bronchiectasis also exhibit a persistent neutrophilic activation in the airways and have higher levels of sputum NE and other inflammatory mediators in the airways than healthy subjects (25). In our study, neutrophil dominance was detected in all sputum (100%) of patients with bronchiectasis. Also, blood neutrophil count (p=0.026) and CRP value (p=0.003) were found to be statistically significantly higher and blood lymphocyte count (p<0.001) lower in patients with bronchiectasis than the control group.

According to our literature review, there are few previous studies on serum levels of NE. Most publications have investigated sputum neutrophil elastase. Our study was conducted with sputum NE, serum NE, IL-1β, IL-8 and desmosin in patients with bronchiectasis; and this is the most comprehensive study investigating the disease duration, exacerbation frequency, frequency of hospitalization, CRP levels, relations with WBC, neutrophil and lymphocyte counts, whether they vary according to the number of affected lobes and segments and bronchiectasis types, whether they differ according to their etiology, whether they are affected by the history of smoking, whether they vary according to the bacteria that reproduce in the sputum and correlations of these biomarkers with each other.

In our study, serum NE levels were found to be statistically significantly higher in patients with bronchiectasis than in the control group (p<0.001). The diagnostic value of blood NE level in predicting bronchiectasis was analyzed by ROC analysis. When the cut-off value was taken as 12.70 ng/mL; it was observed that it had high sensitivity (71.7%), specificity (77.8%), positive predictive value (76.7%) and negative predictive values (72.9%). A statistically significant positive correlation was found between the blood NE level and the number of exacerbations in bronchiectasis patients (p=0.032, r= 0.316). A statistically significant positive correlation was found

between blood NE level and sputum NE level (p= 0.000, r= 0.982).

IL-1β was found to be much more expressed in the airways of children with prolonged bacterial bronchitis (USBB) and bronchiectasis, a precursor to bronchiectasis in some children, compared to the control group, and this was significantly associated with clinical outcomes. This observation has been found not only in children, but also in adults with chronic respiratory disease, where elevated IL-1β is associated with poor lung function and the presence of pathogenic bacteria in non-CF bronchiectasis (26). Chen et al. reported that IL-1β may be a therapeutic target in these conditions, given that high IL-1β concentration is highly correlated with symptom severity, disease recurrence, and intensity of airway inflammation (27). In our study, IL-1β level was found to be statistically significantly higher in patients with bronchiectasis than in the control group (p<0.002). Similar to previous clinical studies, a statistically significant positive correlation was found between IL1-β and the number of exacerbations (p= 0.036, r= 0.310). ROC analysis was performed to determine the diagnostic value of blood IL-1β level in predicting bronchiectasis disease. When the significant cut-off value was taken as 2,935 pg/mL, high sensitivity (73.9%), specificity (71.1%), positive predictive value (72.3%) and negative predictive values (72.7%) were found similar to NE.

Excessive lung elastin degradation results in increased blood levels and urinary excretion of elastin-derived peptides such as desmosine and isodesmosine (28). Desmosin is a structural amino acid that is specifically released into the circulation when mature elastin is broken down (29). In our study, blood desmosine level was found to be significantly higher in patients with bronchiectasis than in the control group (p<0.001). In the analysis made with ROC analysis, when the cut-off value was 0.505 ng/mL; sensitivity was 67.4%, specificity was 62.2%, positive predictive value was 64.6%, and negative predictive value was 65.1%. In the study of Chalmers et al., desmosine level was correlated with sputum elastase (r = 0.42; P<0.0001). In this study, it was reported that blood desmosine was a good marker of sputum elastase activity and was confirmed to be associated with severe exacerbations of bronchiectasis (19). In our study, in addition to a statistically significant positive and excellent correlation between blood desmosine level and sputum neutrophil elastase (p<0.001, r=0.922), there was also a statistically significant positive excellent correlation between desmosine and blood NE (p<0.001, r=0.944). At the same time, a statistically significant positive correlation (p= 0.026, r= 0.328) was found between the desmosine level and the number of exacerbations. Polverino et al. reported that because exacerbations are a very important prognostic factor in bronchiectasis, desmozin can be used to select patients with frequent exacerbations who may benefit from antielastase therapy (30).

Overexpression of IL-8 in the bronchial epithelium has been shown to benefit lung immunity against bacterial infection, but specifically leads to impaired lung function by causing lung injury through persistent inflammation, lung remodeling, and damaged tight connective tissues (13). In the study of Ayhan et al., the serum IL-8 level of the patient group in patients with stable bronchiectasis was found to be statistically significantly higher than the control group (P=0.001). In this study, it was reported that IL-8, an inflammatory cytokine, was found to be correlated high in both serum and BAL. These significant findings and correlation suggested that bronchial inflammation continued in patients who were not in the exacerbation period. This has led to the thought that bronchial tissue damage continues in different localizations and in different intensities as long as the inflammation continues. Although the physiological significance of high IL-8 levels in the stable period of bronchiectasis cases is not fully known, it was reported to support the fact that inflammation continues and the systemic cellular response is active even in stable periods (31). Bergin et al. detected high levels of IL-8 in airway samples taken from patients with bronchiectasis without cystic fibrosis. It was stated that the presence of high IL-8 levels supports the use of appropriate anti-inflammatory therapies (32). In our study, blood IL-8 levels were higher in patients with bronchiectasis than in the control group (173.58±187.13 - 116.95±101.44, p=0.284), but no statistically significant difference was found. Angrill et al. showed that airway inflammation is persistent in patients with bronchiectasis, and NE and other inflammatory mediators were higher in the bronchoalveolar lavage (BAL) of 23 patients, even in the absence of bacterial colonization, compared with healthy subjects (33). Previous studies have reported that purulent sputum is associated with NE concentration and can be considered as a marker for proteolytic and inflammatory activity. Moreover, NE has been shown to increase progressively with increasing bacterial load in sputum. Chalmers also reported a strong association between sputum

bacterial load and a range of inflammatory mediators in a cohort of 434 bronchiectasis patients (25). In our study, 37 of the patients with bronchiectasis had the complaint of active sputum production, and the mean NE value in the sputum sample of these 37 patients was measured as 31.68±17.00 ng/mL. In other biomarkers examined by sputum neutrophil elastase level; a statistically significant and excellent positive correlation was found between blood NE, desmosine and IL-1 beta. There was no significant correlation between sputum NE and bacterial load and type, probably due to the low number of patients who could give sputum samples and the low number of bacteria grown in sputum. In our study, when sputum NE levels were compared according to bronchiectasis type, it was found that patients with cystic bronchiectasis (37.52±18.86 ng/mL) were higher than those with tubular bronchiectasis (26.14±13.26 ng/mL). It was not statistically significant, but it had a p value very close to significance (p=0.054).

Besides, in our study, a statistically significant correlation was found between the number of exacerbations and the number of affected lobes and segments in the patient group with bronchiectasis (p<0.001). Again, a statistically significant correlation was found between the number of affected lobes and segments and the number of hospitalizations (p<0.001, p=0.003, respectively). These relationships show that the number of affected lobes and segments of the patient increases as the number of exacerbations and the number of hospitalizations increase, in accordance with Cole's vicious cycle in the pathogenesis of bronchiectasis, while the frequency of exacerbations and hospitalizations increases as the involved lung section increases (34).

COCLUSIONS

In our study, it was observed that NE, IL-1β and desmosine in the blood of patients with bronchiectasis were significant inflammatory markers that were significantly higher than the control group, had significant diagnostic value, and could be an important component of the diagnosis and follow-up parameters since these parameters were significantly correlated with the frequency of exacerbations. The excellent positive correlation of NE in sputum with these three biomarkers suggested that it may be a strong candidate for routine follow-up parameters. In particular, blood NE and IL-1β stand out as very valuable parameters that should not be ignored in the follow-up of the disease, with the highest sensitivity, specificity, NPD and PPD values. Further studies are needed to evaluate the contribution of blood NE, IL-1β and desmosine in determining the etiology of bronchiectasis, its relationship with symptoms, its relationship with bacteria isolated in sputum culture, and their correlation with bronchiectasis types.

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