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Original Article / Özgün Araştırma

Serum Interleukin-37 Levels in Patients with Systemic Sclerosis and its Relation with Clinical Findings

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Abstract

Aim: This study investigates serum interleukin(IL)-37 levels in patients with systemic sclerosis (SS) and assesses its relationship with clinical findings.

Methods: This study included 35 patients with SS and 30 healthy control subjects. The demographic and clinical characteristics of the patients, such as the presence of Raynaud's phenomenon, SS subtype, digital ulcers, gastrointestinal and lung involvement, and disease activity, were recorded. The medications used by the patients were recorded, and Serum IL-37 levels were measured using an enzyme-linked immunosorbent assay. The United Kingdom Functional Scoring system was used to evaluate the functional status of the patients, while the Valentini criteria were used to evaluate disease activity. Skin involvement was evaluated based on the modified Rodnan skin score.

Results: Although serum IL-37 levels were found to be lower in patients with SS than in the control group, the difference was not statistically significant (p= 0.078). A negative correlation was identified between serum IL-37 levels and C3 levels in patients with SS (p= 0.046). No significant relationship was found between IL-37 levels and other clinical and laboratory parameters.

Conclusion: Unlike in patients with autoimmune disorders, serum IL-37 levels were found to be lower in patients with SS than in the control subjects, and IL-37 demonstrated a negative correlation with C3 levels.

Keywords: Systemic sclerosis, Interleukin-37, complement

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Sistemik Sklerozis Hastalarında İnterlökin-37 Serum Düzeyi ve Klinik Bulgularla İlişkisi

Öz

Amaç: Sistemik skleroz (SS)' lu hastalarda serum interlökin (IL)-37 düzeyi ve klinik bulgularla ilişkisinin araştırılması amaçlandı.

Yöntemler: Otuz beş SS hastası ve 30 sağlıklı kontrol çalışmaya dahil edildi. Hastaların demografik özellikleri ve Raynaud fenomeninin varlığı, SS alt tipi, dijital ülserler, gastrointestinal ve akciğer tutulumu gibi klinik özellikler ve hastalık aktivitesi kaydedildi. Hastaların kullanmış oldukları ilaçlar kaydedildi. Serum IL-37 düzeyleri enzyme-linked immunosorbentassay ile ölçüldü. Hastaların fonksiyonel işlevlerinin değerlendirilmesinde United Kingdom Fonksiyonel Skorlaması kullanıldı. Hastalık aktivitesinin değerlendirilmesinde ise Valentini kriterleri kullanıldı. Deri tutulumu Modifiye rodnan skoru ile değerlendirildi.

Bulgular: SS hastalarının serum IL-37 düzeyleri kontrol grubuyla karşılaştırıldığında daha düşük olmasına rağmen istatistiksel olarak anlamlı değildi(p=0.078). SS hastalarının serum IL-37 düzeyleri ile C3 düzeyi arasında negatif korelasyon saptandı (p=0.046). IL-37 ile diğer klinik ve laboratuar parametreleri arasında anlamlı ilişki bulunmadı.

Sonuç: IL-37 düzeyi, SS hastalarının serumlarında kontrollerle karşılaştırıldığında diğer otoimmün hastalıkların aksine düşüktü ve C3 düzeyi ile negatif korelasyon gösterdi.

Anahtar kelimeler: Sistemik sklerozis, interlökin-37, kompleman.

INTRODUCTION

Systemic sclerosis (SS) is a chronic autoimmune and inflammatory disorder that is characterized by fibrosis of the skin and various internal organs[1]. The etiology and pathogenesis of SS are not yet fully understood, although genetic predisposition, environmental factors, infections and microchimerisms are considered as possible triggers of the pathogenetic process[2].

The pathogenesis of scleroderma consists of a triad of vasculopathy, immune activation and fibrosis[3]. The innate and acquired immune systems are reported to play a key role in the pathogenesis of scleroderma, along with a complex series of factors that involve immune activation, vascular damage and fibrosis, with cytokines shown to be elevated in SS and involved in many steps[1,3,4].

IL-37 is a recently discovered member of the IL-1 family that is defined as a natural inhibitor of immune response[5]. IL-37 has been identified as an anti-inflammatory cytokine in various autoimmune/inflammatory disorders[6]. While the relationship between the IL-1 family and SS has been subjected to a general evaluation, there is as yet insufficient specific data on IL-37.

The present study aims to determine serum IL-37 levels in patients with SS which is an inflammatory and autoimmune disorder that is characterized by fibrosis throughout its course and also to identify possible relationships within the clinical findings.

METHOD

Included in the study were patients who presented to the Rheumatology outpatient clinics of the Department of Physical Medicine and Rehabilitation at Dicle University Faculty of Medicine Hospital between October 2016 and January 2017, and who met the ACR/EULAR 2013 scleroderma classification criteria[7], along with matched healthy control subjects. The approval of Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee was obtained before starting the study(07/10/2016/315). All participants were informed of the aim of the study and blood withdrawal, and written consent was obtained for participation.

Patients aged 18–65 years diagnosed with systemic sclerosis according to the 2013 ACR/EULAR classification criteria were included in the study while those diagnosed with a rheumatologic disease other than SS, patients with diabetes mellitus, severe heart failure and significant impairment in hepatic-renal functions and those with infection or malignancy were excluded.

Clinical Evaluation

The sociodemographic characteristics of the patients and the controls were recorded, along with the calculated body mass index (BMI). The date of symptom onset and the time since diagnosis, symptoms related to organ and system involvement and the therapies they had received at the time of enrolment as participants in the patient group were recorded on a Systemic Sclerosis Data Collection Form. Patients who received corticosteroids were classified according to the doses they had received as low dose(<7,5 mg prednisone or equivalent), moderate dose(7,5–30 mg prednisone or equivalent) or high dose(>30 mg prednisone or equivalent). The findings of physical examinations related to organ involvement were recorded and finger-palmar flexion distances were measured. A respiratory function test was performed to identify any pulmonary involvement. High-resolution computed tomography(HRCT) scans that were acquired recently as part of a control program in patients that were placed in a follow-up program or HRCT scans from within the last six months were retrieved from the hospital's database. HRCTs were obtained from patients with abnormal findings identified recently from a posterior-anterior chest x-ray. A transthoracic echocardiography(ECHO) was performed and pulmonary arterial pressure (PAB) was recorded. The functional status of the patients was evaluated using the United Kingdom(UK) Functional Scoring system, disease activity was evaluated based on the Valentini criteria and skin involvement was evaluated using a modified Rodnan skin score(mRSS)[8,9].

Laboratory Evaluation

All of the participants in the patient and the control groups provided an 8–10 ml blood sample from the brachial vein into a biochemistry tube containing no anticoagulant. Sera were obtained by centrifuging the blood samples at 4000 rpm for 1 minute at + 4 0C, and the sera were stored at –200C until analysis. The IL-37 levels in the sera were measured with ELISA using a human IL-37 kit(SunRed Biotechnology Company, Shanghai, China) within the laboratory. Complete blood counts, erythrocyte sedimentation rates(ESR), C-reactive protein(CRP) levels, biochemistries, including liver and kidney function tests and complement level(C3, C4)were obtained at each control visit. The autoantibody panels of the patients were retrieved from hospital records.

Statistical Analysis

All data was analyzed using the SPSS 21.0 software package. A Kolmogorov-Smirnov Test was used to test for the normal distribution of the data and between-group comparisons were made with an Independent samples t-test or a Mann-Whitney U-test, depending on the distribution of the normality of the data. The difference between the proportional data was calculated with a Chi-square test. Considering the fitness of the data to normal distribution, a Pearson's or Spearman correlation analysis was carried out to evaluate the correlation between IL-37 and laboratory and clinical parameters related to SS. In all analyses, a p-value of ≤ 0.05 within a 95% confidence interval was considered statistically significant.

RESULTS

The demographical characteristics and laboratory findings of the patient and the control groups are presented in Table I. Serum IL-37 levels were lower in the patient group than in the control group, although the

difference was not statistically significant (p= 0.078) (Table I).

Table I: Demographic characteristics and laboratory data of the patient and control groups

Data	SS (Mean±SD)	Control (Mean±SD)	p value
Age (years)	47.4±12.3	46.3±9.7	0.682
Gender (male/female)	3/32	3/27	0.843
BMI (kg/m^2)	26.6±6	27.4±4	0.630
ESR (mm/hr)	17.4±9	11.6±5	**0.002**
CRP (mg/dL)	0.68±0.71	0.35±0.06	**0.011**
IL-37 (pg/mL)	55.1±44.1	73.9±47.8	0.078

BMI:Body Mass IndexCRP:C-reactive proteinESR: Erythrocyte sedimentation rateIL-37:Interleukin-37SD:Standard deviation

The therapies undertaken by the patients at the time of enrolment are shown in Table II. Of the total, two patients had been newly diagnosed and so were under no medication; There 25 patients were receiving corticosteroid therapy, 18 of which were on low doses and the remaining seven were on moderate doses.

The clinical findings of the patients are presented in Table III. Of the 35 patients with SS included in this study, 22 had lcSS, 12 had dcSS and one had sine scleroderma. HRCT scans revealed ground-glass areas in 23 patients, honeycomb appearances in three patients, reticular densities in two patients, subpleural nodules in one patient and normal findings in six patients. FVC was decreased(< 80%) in 12 patients, while PAB on an echocardiography was 40 mmHg or higher in three patients.

A muscle strength examination revealed proximal muscle weakness in three patients. No patients had renal crisis, while eight patients had calcinosis(four had dcSS and four had lcSS).

There were various degrees of limitation in the finger-palmar flexion distance in 15 patients. Of the 35 patients, 14 had active disease(Valentini score ≥ 3) and of these, five were of the lcSS subtype and nine were of the dcSS subtype.

Table IV presents the clinical and laboratory parameters of the patients with limited and diffuse disease.

The relationship between serum IL-37 levels and demographic characteristics, laboratory findings and clinical parameters were also evaluated (Table V).

The serum IL-37 levels were not significantly different between the patients who used steroids and immunosuppressive therapies and those who did not (p= 0.324, p= 0.95, respectively).

Table II: Therapies received by the patients

Therapy	N (%)
Calcium channel blocker	28 (80)
Pentoxifylline	4 (11.4)
Iloprost	2 (5.7)
ACE inhibitors	2 (5.7)
Azathioprine	15 (42.9)
Cyclophosphamide (pulse)	3 (8.6)
Corticosteroid	25 (71.4)
Methotrexate	6 (17.1)
Bosentan	1 (2.9)
Hydroxychloroquine	10 (28.6)
Colchicine	16 (45.7)
Acetylsalicylic acid	16 (45.7)
Mycophenolatemofetil	2 (5.7)
Rituximab	2 (5.7)
Non-steroid anti-inflammatory drugs	5 (14.3)
Proton pump inhibitors	21 (60)

ACE: Angiotensin-converting enzyme

Table III: Clinical findings of the patients

Clinical finding	N (%)
Raynaud's phenomenon	35 (100)
Digital ulcer	11 (31.4)
Digital scar	13 (37.1)
Sclerodactyly	32 (91.4)
Edema	26 (74.3)
Atrophy	11 (31.4)
Telangiectasia	16 (45.7)
Tendon friction rubs	18 (51.4)
Calcinosis	8 (22.9)
Flexion contracture	13 (37.1)
Dysphagia	19 (54.3)
Dyspnea	26 (74.3)
Dyspepsia	29 (82.9)
Vomiting	4 (11.4)
Diarrhea	7 (20)
Constipation	10 (28.6)
Palpitation	11 (31.4)
Angina	7 (20)
Dizziness	10 (28.6)
Proximal muscle weakness	3 (8.6)
Weight loss	12 (34.3)

Table IV: Clinical and laboratory parameters of patients with limited and diffuse disease

Parameter	lcSS n=22	dcSS n=12	p value
Rodnan score (Mean±SD)	8.6±4	17.5±6.5	**0.001**
Functional score (Mean±SD)	8±6.2	16.9±8	**0.004**
Valentini score (Mean±SD)	1.9±1.2	3.4±1.5	**0.011**
FVC (Mean±SD)	73±15.6	69±9	0.353
PAB (mmHg)	24.8 ±8.5	27.9±8.5	0.324
ESR (mm/hr)	18.4±10	16.3±7.2	0.494
CRP (mg/dL)	0.69±0.83	0.68±0.49	0.958
C3 level (mg/dL)	109.73±24.68	110.11 ±19.92	0.969
C4 level (mg/dL)	21.54 ±8.62	21.57 ±4.78	0.992
ACA positivity (n, %)	3 (13.6)	0 (0)	0.537
Anti-scl-70 positivity (n, %)	1 (4.5)	10 (83.3)	**<0.001**
Serum IL-37 level (pg/mL)	54.3±42.7	49.3±42.7	0.74

FVC:Forced vital capacityPAB:Pulmonary arterial pressureCRP:C-reactive protein ESR: Erythrocyte sedimentation rateC: Complement ACA: Anticentromere antibodies IL-37:Interleukin-37SD:Standard deviation

Table V: Relation of IL-37 levels with demographic characteristics and laboratory and clinical parameters

	IL-37	
	p	(r)
Age	0.442	.134
Time since diagnosis	0.932	.015
ESR	0.990	.002
CRP	0.240	.204
C3	**0.046**	**-.441**
C4	0.654	-.104
MRS	0.433	-.137
Functional score	0.586	.095
Valentiniscore	0.960	-.009
PAB	0.399	-.147
FVC	0.129	.262

FVC:Forced vital capacityPAB:Pulmonary arterial pressureCRP:C-reactive protein ESR:Erythrocyte sedimentation rateC:ComplementMRS:ModifiedRodnan score IL-37:Interleukin-37

DISCUSSION

Although serum IL-37 levels were lower in patients with SS than in the control group, there was no statistically significant difference although a negative correlation was identified between IL-37 levels and C3 levels. IL-37 is defined as an anti-inflammatory cytokine that suppresses innate and adaptive immunity, and IL-37 expression is found to be associated with the presence of inflammatory environments and inflammatory cells. It has further been suggested to play a role in autoimmune disorders, suppressing excessive immune response in inflammatory disorders[5,10,11]. Yang et al.[12] reported higher IL-37 levels in patients with RA than in healthy controls, while in the same study, elevated serum IL-37 levels were shown to be positively correlated with inflammatory cytokines(IL-17 / IL-23) and disease activity. In another study, involving 90 patients with Systemic Lupus Erythematosus(SLE), Wu et al.[13] reported significantly higher serum IL-37 levels in patients with SLE than in the control group. In contrast to both of the studies reported above, serum IL-37 levels were found to be lower than in the control group in the present study. The disease specific pathogenesis could be a possible cause of the lower IL-37 levels found in patients with SS. The pathogenesis of SS is

extremely complex; involving vasculopathy, immune activation and fibrosis steps. Vascular damage is generally the trigger event in the pathogenesis while fibrosis becomes involved in the final stage. Inflammation regresses over time and the vascular inflammatory phase is replaced by fibrosis which results in a disruption of tissue structure[2,14].

It should be noted that IL-37 levels are low and not continuously expressed in tissues in which inflammatory stimulation is absent[5,11] and this characteristic in the pathogenesis of SS could be the cause of the lower IL-37 levels found in the present study. Another reason for the lower IL-37 levels in patients with SS when compared to the control group could be related to the direct or indirect relationship between IL-37 and vascular damage in the pathogenesis of SS. In one study[15], IL-37 has been defined as a new proangiogenic factor in developmental or pathological angiogenesis. The study demonstrated that IL-37 supports endothelial cell proliferation and migration and activates survival signaling in endothelial cells and that IL-37 expression in endothelial cells increases in response to hypoxia. IL-37 supports vascular growth and induces developmental angiogenesis in neonatal experimental animals while improving pathological angiogenesis and promoting neovascularization in experimental animals with oxygen-induced retinopathy[15]. That said, apart from vascular damage, angiogenesis is problematic and insufficient in SS[16]. It can thus be understood that decreased IL-37 levels may be a triggering factor in the pathogenesis of SS; causing defective angiogenesis and vascular damage.

Previous studies have reported different results regarding the relationship between IL-37 and C3 levels[10,13]. Ye et al.[10] reported a higher IL-37 expression in patients with active SLE when compared to patients with inactive disease and a control group, and also identified a significant negative correlation between IL-37 and complement levels. Wu et al.[13], however, found higher serum IL-37 levels in patients with SLE when compared to the control group, but unlike in previous studies they reported a positive correlation between IL-37 and C3 levels. The present study identified a negative correlation between IL-37 and C3 levels revealing a need for further studies based on disease-specific pathogeneses to identify the nature of the relationship between IL-37 levels and its complements.

Bouali et al.[17] reported significantly lower IL-37 levels in patients with Behcet's disease than in the healthy controls, and after separating the patients with Behcet's disease according to disease activity, they reported significantly lower IL-37 levels in patients with active disease than in patients with inactive disease. These results indicate that decreased IL-37 production may be correlated with a disease in activity in Behcet's disease. Although IL-37 levels were lower in patients with SS when compared to the control group, the difference was not statistically significant, and this may be a result of the small sample size in the present study.

CONCLUSION

The present study identified lower IL-37 levels in patients with SS than in the control group, although the difference was not statistically significant. A significant negative correlation was identified between IL-37 and C3 levels. Albeit statistically insignificant, the decreased IL-37 levels may be associated with the fact that cytokine promotes vascular damage and immune activation, being regarded as the main inhibitor of innate immunity and considered to play a role in angiogenesis. Prospective studies evaluating IL-37 levels involving a larger number of patients with SS could contribute to the elucidation of this relationship.

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Ethics Committee Approval: The approval of Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee was obtained before starting the study (07/10/2016/315).

Conflict of Interest: The authors declared no conflicts of interest.

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