Sistemik Sklerozlu Hastalarda ve Sağlıklı Bireylerde Serum IL-23 ve IL-17 Seviyelerinin Karşılaştırılması

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Abstract

Aim: Systemic Sclerosis (SSc) is a chronic autoimmune-multisystemic disease with unknown aetiology. Fibrosis, vasculopathy and immune system activation are responsible for SSc pathogenesis. Cytokines such as interleukin (IL)-17 and IL-23 may play a role in disease development, disease severity, involvement and characteristics rather than one. For this reason, we aimed to compare the serum levels of IL-17 and IL-23 in SSc patients and healthy controls. Material and Method: Forty patients with SSc and forty healthy control were included in the study. Autoantibody profiles, organ involvement of the patients were determined. Modified Rodnan skin scores (MRSS) of the patients were calculated. Serum IL-17 and IL-23 levels were measured by ELISA. Results: The patients had a mean MRSS of 13.15. Any statistically significant difference between MRSS and IL-17/IL-23 levels were not detected. (p=0.142 and p=0.668, respectively). Twenty-one (52.5%) patients had lung involvement and 16 (40%) patients had gastrointestinal involvement. Thirty-six (90%) patients were ANA positive, 21 (52.5%) patients were anti-Scl-70 positive, 6 (15%) of the patients were anti-centromere positive. The mean IL-17 levels of the patients and control group were anti-Scl-70 positive, 6 (15%) of the patients were anti-centromere positive. The mean IL-23 levels of healthy control group and patients were found to be 30.27±12.68 pg/ml and 29.32±10.52 pg/ml (p=0.007), respectively. The mean of IL-23 levels of healthy control group and patients were found to be 30.27±12.68 pg/ml and 29.32±10.52 pg/ml (p=0.007), respectively. There were no statistically significant relation between serum levels of IL-17/IL-23 and age, pulmonary and gastrointestinal involvement, disease severity, autoantibody profiles. The levels of IL-17 differed significantly in SSc patients and control group but not so in IL-23. Discussion: In our study we concluded that SSc is associated with IL-17, but not with IL-23. IL-17 might contribute to SSc pathogenesis, but no correlation was found between the serum levels of IL-17/IL-23 and clinical symptoms, laboratory findings or disease activity. Anti-IL-17 can be a potential therapeutic agent in SSc patients.

Keywords

Interleukin-17, Interleukin-23, Systemic Sclerosis

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Introduction
SSc is a complex disease and remains incompletely understood. There is immune activation, vascular damage, and excessive synthesis of extracellular matrix with deposition of increased amounts of structurally normal collagen which are important in the development of this illness. Due to accumulation of fibrous connective tissue, internal organ involvements such as gastrointestinal system, lung, heart, and kidney can be seen in this disease. Fibrosis, vasculopathy and immune system activation are responsible for its pathogenesis [1,2].

Inflammation due to the immune activation characterized with T cells, macrophages, mast cells and rarely B cells may be observed in early phase skin lesions patients with systemic sclerosis [3]. Although mononuclear cells consist of mainly CD4+ T cells and macrophage in the early affected skin areas, CD8+ T cells can be found dominantly in lungs. CD4+ T cells have cytokine profiles characterizing Th2, such as IL-4, IL-10, IL-13, IL-17, IL-23 [4]. Another T cell subtype is Th17 which is different from Th1 and Th2. When Th17 cells are activated by IL-23, they secrete IL-17, so IL-23 is important for stabilizing Th17 cells. It was shown that IL-17 is elevated in tissue and peripheral blood of SSc patients. IL-17 can activate fibroblasts and fibroblasts secrete proinflammatory cytokines, such as IL-6 and IL-8. When IL-17 stimulates macrophages, they produce TNF-α and IL-1. Accordingly, endothelial cells can be stimulated by IL-17, and they secrete IL-1, IL-6 of which causing elevation of ICAM-1 and VCAM-1 levels [5]. There are some proofs that Th17 cells and associated cytokines play roles in some disease and infections such as; psoriasis [6], rheumatoid arthritis [7], multiple sclerosis [8], inflammatory bowel diseases, asthma and some bacterial, fungal infections [9,10].

IL-17 and IL-23 levels in SSc patients were investigated in some studies: Kurusawa K et al and Murata M et al, found higher IL-17 levels in SSc patients compared to healthy controls in their studies [11, 12]. Komura K et al, found higher IL-23 levels in SSc patients [13]. In another study by Radstake TR et al, found both higher IL-17 and IL-23 levels in SSc patients [14]. However, IL-17 and IL-23 levels were determined higher in healthy controls compared to SSc patients in some studies [15]. Also, there have been studies addressing the possible relationship between IL-17, IL-23 and organ involvement, disease duration, autoantibody positivity. Controversial results were found in few studies. SSc, which is a chronic autoimmune disease, the roles and manner of IL-17 and IL-23 have not been fully understood. This issue is important in understanding of SSc pathogenesis and innovating targeted therapies for patients with SSc. If researchers understand and show the importance of IL-17 and IL-23, they could solve a big puzzle.

So, we aimed to compare the serum IL-17 and IL-23 levels in patients with SSc and healthy individuals. We investigated the relationship between clinical and autoantibody subset profiles of SSc patients and the levels of serum IL-17 and IL-23.

Material and Method
SSc patients who have been followed in the inpatient clinic and/or outpatient clinic of Ankara University Faculty of Medicine, Department of Rheumatology and healthy volunteers with similar age and gender to the patient group, no known disease and no-use of any drugs were enrolled in the study. According to American College of Rheumatology (ACR) diagnostic criteria [16], 40 patients (35 female, 5 male) diagnosed with SSc and control group of 40 healthy subjects (35 female, 5 male), were included to the study. Ankara University Faculty of Medicine Ethics Committee Approval, dated 28.06.2010 and decision number -13-264, was obtained.

Inclusion Criteria
1) Patients who are diagnosed systemic sclerosis according to the ACR diagnostic criteria [16].

Exclusion Criteria
1) Patients or the healthy volunteers that have different additional diseases (such as any malignancy and autoimmune diseases and have previously identified/known autoantibody positivity (RF, ANA, ANCA, etc).
2) Patients with severe psychiatric disorders that could affect cooperation (especially in the last 30 days in the treatment of psychosis and depression).

Recent and previous data from patient files were reached. Modified Rodnan skin scores (MRRS) were calculated for all patients, and patients were divided into groups by previously determined method [17] according to skin involvement, whether patients with diffuse cutaneous systemic sclerosis (dcSSc) or limited cutaneous systemic sclerosis (lcSSc). Informed consents were obtained from all subjects. After centrifugation serum samples stored at -20oc until study-day. Serum IL-17 and IL-23 levels in patients and control group were studied by eBioscience Platinum ELISA kit® (USA) in Immunology Laboratory of Ankara University Faculty of Medicine.

50 μl of standard and serum samples were added to the wells. Then 50 μl of biotinylated antibodies were added to each well, and incubated. Two hours later, incubation solutions were washed and 100 μl solution of streptavidin-HRP were added and incubated at room temperature for one hour. Then the standard curves were calculated at 450 nm spectrophotometer by ELISA technique. Accordingly, the minimum detectable level of IL-17 was 0.5 pg/ml. IL-17 level in serum of patients and controls were calculated as described above. The IL-17 levels of control groups and patients were entered as 0.5 pg/ml into SPSS program.

The IL-23 levels of the groups was calculated like IL-17 as mentioned previously. Accordingly, the minimum detectable IL-23 level was 10 pg/ml. The IL-23 levels of control group and patients were entered as 10 pg/ml into SPSS program.

Statistical Analysis
The sample size of the study was calculated with G*Power (G*Power Ver. 3.0.10, Franz Faul, Universität Kiel, Germany). The required sample size for 95% power, α=0.05 Type I error, β=0.05 Type II error and d=0.35 effect size was calculated as min 79 subject total. So the authors decided to enroll minimum 40 subjects in each group.

For registration and statistical analysis of data, SPSS (Statistical Package for Social Sciences, Chicago, IL 60606, USA) 16.0 software package was used. Descriptive statistical data are presented as the mean ± standard deviation (median, minimum-
maximum). Kolmogorov-Smirnov and Shapiro-Wilk normality tests were performed for suitability of the normal distribution of data. Parametric tests were used for the evaluation of the data complying with normal distribution whereas non-parametric tests were used for the evaluation of the data that do not comply with normal distribution. Skewed data are shown as the median and range. Univariate comparisons between nominal variables were performed using the chi-square test. The Mann-Whitney U test was used for non-normal variables. The Kruskal-Wallis non-parametric analysis of variance was used for comparison of three groups. A p value <0.05 was considered as statistically significant.

Results

The mean age of the patient group was 46.5 ± 14.4 (44.5, 20-76) years. The mean disease duration was 7.7 ± 6.95 (5.4, 1-28) years. The median disease duration of dcSSc was 10 (24, 1-25) years. The median disease duration of lcSSc was 4 (27, 1-28) years. Of the 40 patients, 16 (40%) were dcSSc and 24 (60%) were lcSSc. The mean age of the dcSSc group was 49.44±13.65 years, and the mean age of the lcSSc group was 44.5 ± 15 years. Of 40 controls, 5 (12.5%) of the healthy volunteers were male and 35 (87.5%) were female. In the control group, mean age was 40.2 ± 10.7 (38, 26-74) years. There was no statistically significant difference between the groups according to age (p = 0.058) and gender (p > 0.05).

For all SSC patients, the mean MRSS was 13.15±6.14. Twenty eight (70%) patients had Raynaud’s phenomenon. Lung involvement in 21 (52.5%) of the patients, and gastrointestinal tract involvement in 16 (40%) of the patients were detected (Table 1). ANA positivity was observed in 36 (90%) of the patients, and the others were as follows; 21 (52.5%) of anti-Scl-70 antibody-positive, 6 (15%) of anti-centromere antibody positive, 4 (10%) of U1-RNP antibody positive, 11 (27.5%) of anti-SS-A antibody positive, and 3 (7.5%) of anti-SS-B antibody positive (Table 1). The mean IL-17 level of the patients was 0.67±0.55 (0.5, 0.5-3.5) pg/ml, and the mean IL-17 of the controls was 0.52±0.10 (0.5, 0.5-1.1) pg/ml. Statistically significant difference was detected between the groups for IL-17 levels (p= 0.007, Mann-Whitney U test). The mean IL-17 level was 0.70±0.46 (0.53, 0.5-2.2) pg/ml. The mean IL-17 level was 0.65±0.61 (0.5, 0.5-3.5) pg/ml in lcSSc, and 0.52±0.10 pg/ml in controls (p< 0.05). The mean IL-23 levels of the groups were as follows 29.46±13.67 (24.36, 12.64-66.26) pg/ml in dcSSc, 29.22±8.10 (3.29, 16.18-49.73) pg/ml in lcSSc, and 30.27±12.68 pg/ml in control (p= 0.65) (Figure 1). The mean IL-17 level of the 21 patients with lung involvement was 0.66±0.40 (0.50, 0.5-2.22) pg/ml, and the mean IL-23 level was 29.71±11.71 (29.03, 16.80-66.26) pg/ml. In 19 patients without pulmonary involvement, the mean level of IL-17 was 0.68±0.69 (0.5, 0.5-3.54) pg/ml, and the mean IL-23 level was 28.88±9.35 (29.3, 12.64-49.73) pg/ml (Figure 1). There was no statistically significant difference detected between the groups (p=0.138 and p=0.936, respectively). The mean IL-17 level of 16 patients with gastrointestinal involvement was 0.55±0.1 (0.5, 0.5-0.84) pg/ml, and the mean IL-23 level was 31.82±12.58 (30.53, 16.8-66.36) pg/ml. In 24 patients without gastrointestinal involvement, the mean level of IL-17 was 0.75±0.7 (0.5, 0.5-3.54) pg/ml, and the mean IL-23 level was 27.65±8.78 (26.5, 12.64-49.73) pg/ml. There was no statistically significant difference detected between the groups (p= 0.52 and p= 0.279, respectively). The relationship between the disease duration and IL-17, IL-23 levels was not detected (p= 0.071 and p= 0.257, respectively). There was not any statistically significant difference between IL-17 and IL-23 levels in terms of MRSS (p= 0.142 and p= 0.668, respectively).

In 36 patients with positive ANA, the mean IL-17 level was 0.60±0.31 pg/ml, and IL-23 level was 29.86±10.87 pg/ml. In ANA negative patients, the mean IL-17 level was 1.26±1.52 pg/ml, and IL-23 level was 5.0±24.49 pg/ml, respectively. There was no statistically significant difference between the groups (p= 1.0 and p= 0.207, respectively). In 21 patients with positive Anti-Scl-70, the mean IL-17 level was 0.66±0.40 pg/ml, and IL-23 level was 29.07±11.70 pg/ml. In Anti-Scl-70 negative patients, the mean IL-17 was 0.68±0.69 pg/ml, and IL-23 level was 29.59±9.36 pg/ml. However, no statistically significant differ-
ences were found between the groups (p= 0.789 and p = 0.810, respectively). In Anti-centromer positive-patients, the mean IL-17 was 0.53±0.07 pg/ml, and IL-23 level was 32.48±5.91 pg/ml. In Anti-centromer negative-patients, the mean IL-17 was 0.69±0.59 pg/ml, and IL-23 level was 28.76±11.11 pg/ml. There was no statistically significant difference between groups (p= 0.897 and p= 0.137, respectively).

Discussion

Primary function of Th17 cells is to eliminate the pathogens that can not be cleared effectively by Th1 and Th2 cells. For this purpose, Th17 cells are rapidly recruited in the inflammation sites, and acting as a bridge between natural and acquired immunity. They recruit other T helper cells. Th17 cells are one of the main triggers of tissue inflammation, and they were shown to be involved in many experimental autoimmune diseases, and various inflammatory situations [18]. Many recent studies suggest that Th17 cells are “sine qua non” in autoimmune diseases. IL-23 regulates the maturation of self-reactive IL-17-producing T cells, and with the help of IL-17, IL-6, IL-8 and TNF-alpha, triggers chronic inflammation associated with neutrophil and macrophages [19]. IL-17 producing T cells were isolated and IL-23 was shown to be effective in proliferation of these cells [20]. SSc is an autoimmune, multi-systemic disease of unknown etiology. Autoimmunity, and uncontrolled fibroblast activation causing vascular damage and tissue fibrosis are thought to be responsible for the pathogenesis. SSc is generally assumed to be a Th2 cytokine disease [15]. Th1 cytokines, such as interferon gamma, have anti-fibrotic effects while Th2 cytokines, such as IL-4 and IL-13, have profibrotic effects [15]. These CD4+ T cells have a Th2-like cytokine profile, secreting cytokines like IL-4, IL-10, IL-13, IL-17, IL-23. IL-17, originally named cytokotic T lymphocyte antigen 8 (CTLA-8) is a 17 kDa type-1 transmembrane protein. IL-17 activates fibroblasts, inducing them to secrete proinflammatory cytokines like IL-6 and IL-8, upregulates ICAM-1 expression on the surface, and stimulates endothelial cells to secrete IL-6. IL-23 is a heterodimeric cytokine consisting of p19 and p40 subunits. IL-23 is involved in the development of Th17 cells, a distinct T cell subtype characterized by its IL-17 secretion [21].

Early studies had focused on animal models of experimental autoimmune encephalitis and rheumatoid arthritis and shown the beneficial effects of IL-17 blockage and inhibition of IL-17 producing cells [22,23]. Also, anti-IL-23(p19) treatment was shown to cure mice with inflammatory bowel disease [23]. By producing certain chemokines and cytokines, Th17 cells trigger organ-specific destructive inflammatory processes. Some studies on SSc patients had suggested that IL-17 and IL-23 take part in disease pathogenesis with their potent proinflammatory capacity. In this respect, anti-IL-17 and anti-IL-23 targeted therapies may be developed and be beneficial for SSc patients with different organ involvements. So, different randomized control trial designs can be made for evaluating this effect.

In the literature, there are various studies demonstrating that IL-17 and IL-23 levels are elevated in SSc patients compared to healthy controls. There are also studies on the relationship of IL-17 and IL-23 levels and organ involvement, disease duration and antibody positivity. In our study, we compared the IL-17 and IL-23 levels of SSc patients and healthy controls; distribution of IL-17 and IL-23 levels in diffuse and limited subtypes, and the correlation of IL-17 and IL-23 levels with organ involvement, disease duration and antibody positivity. We measured IL-17 and IL-23 levels of patients and control group that showed no statistically significant difference between age and gender distribution. In the patient group, mean IL-17 levels were found to be significantly higher than the healthy controls (p = 0.007). While mean IL-23 levels were lower in the patient group compared to healthy controls, and no statistically significant difference was detected (p= 0.60).

In a study conducted by Kurusawa et al., IL-17 levels were found to be significantly higher in SSc patients compared to SLE patients and healthy controls. In SSc patients, it was demonstrated that IL-17 production was increased in the fibrotic lesions of lung and skin, and in stimulated peripheral blood lymphocytes [11]. In the study conducted by Murata et al. including 59 patients and 15 healthy controls, IL-17 levels were found to be significantly higher in the patient group compared to control group [12]. In the study by Komura et al., involving 63 SSc, 15 SLE patients and 31 healthy controls, IL-23 levels were found to be significantly higher in SSc patients compared to SLE patients and healthy controls [13]. In contrast to our study, in a study by Radstake et al., along with increased IL-17 levels in the patient group, had also found IL-23 level and IL-23 receptor expression on CD4+ cells to be higher comparing to the control group was shown in a study by Radstake et al. [14]. In contrast, in a study by Gourh et al. on 444 SSc and 216 healthy controls, IL-17 and IL-23 levels were found to be lower while IL-6, IL-13 ve TNF alpha levels were higher in the SSc group [15].

We found no statistically significant difference between serum IL-17 and IL-23 levels in dcSSc and lcSSc groups (p= 0.066 and p= 0.652, respectively), supporting the findings of Murata et al. [12], as they could not find any difference between IL-17 levels of dcSSc and lcSSc [12].

In SSc pathogenesis, immune system dysregulation is thought to take part especially during the earlier stages [15]. In our study, no statistically significant difference was detected between disease duration and IL-17 and IL-23 levels in the non-parametric correlation analysis (p= 0.071 and p= 0.257, respectively). These results are similar to Murata et al., regarding IL-17 levels [12]. On the other hand, Gourh et al., showed that as the disease progresses, IL-17 levels decrease, and IL-23 levels increase in the second decade after diagnosis [15]. Kurusawa et al. also stated that IL-17 had an important role in SSc pathogenesis [11].

No statistically significant difference was detected between the MRSS (which demonstrates skin involvement, disease severity) and IL-17 and IL-23 levels using the non-parametric correlation analysis (p= 0.071 and p= 0.257, respectively). These results are similar to Murata et al., regarding IL-17 levels [12].

In our study no correlation was found between ANA, anti-Scl-70 and anti-centromere antibody positivity and serum IL-17 and IL-23 levels. In the study conducted by Gourh et al. [15], IL-17
levels were significantly higher in antibody negative group comparing to Anti-Scl-70, Anti-centromere antibody or Anti-polymerase antibody positive group. On the other hand, IL-23 levels, were more elevated in the anti-Scl-70 antibody positive group [15]. The study by Murata et al. also supports these findings, as IL-17 levels were found to be lower in anti-Scl-70 positivity [12]. Recent studies have shown that IL-17 and Th17 cells might have a role in SSc development and progression [23]. IL-1a, IL-6 and TGF-β are required for the differentiation of naïve T cells to Th17. In contrast, IL-23 is required for the stabilization of the Th17 phenotype [23,24]. Although some studies demonstrated that IL-17 and IL-23 levels are high in SSc patients, other studies have not supported these findings. In our study, IL-17 levels of the SSc patients were found to be significantly higher than those of healthy controls, however, there was no statistically significant difference between the serum IL-23 levels of patients and healthy controls was detected. Moreover, serum levels of both of these cytokines were found not to be correlated with clinical and laboratory findings of SSc.

In conclusion, compared to similar studies in the literature, the number of patients in our study can be considered sufficient. IL-17 and IL-23 might contribute to systemic sclerosis pathogenesis, but no correlation was found between the serum levels of these cytokines and clinical symptoms, laboratory findings or disease activity. Further studies, powered to involve more patients with clinically active disease are required to prove our hypothesis. Also, we concluded that SSc is associated with IL-17, but not with IL-23. Also in our study, the investigation of IL-17 and IL-23 levels, the studies are propagated regardless of the patients’ treatment. The limitations of the present study include the lack of assessment of treatment modalities, drug usage and the effects of the drugs on the serum IL-17 and IL-23 levels. These factors may have also affected the results. To eliminate the difference between studies, the role of cytokine on etiopathogenesis in treated and untreated patients should be inquired. Serum IL-17 and IL-23 levels in SSc patients may also be affected depending on whether the patients use immunosuppressive therapy or not. Further studies are necessary for evaluating these relations.

Competing interests

The authors declare that they have no competing interests.

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