Serum interleukin 23 and its associations with interstitial lung disease and clinical manifestations of scleroderma

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Introduction Systemic sclerosis (SSc) is a complex disease linked to immune system activation, vascular damage, associated with increased synthesis, and deposition of extracellular matrix, which contain excessive amounts of structurally normal collagen. Interleukin 23 (IL-23) might play a role in disease development and severity. This study aimed to assess the relationship between serum level of IL-23 and interstitial lung disease in SSc.

Patients and methods Thirty patients with SSc together with 30 age-matched and sex-matched healthy volunteers were recruited in this study. Serum IL-23 levels were measured by enzyme-linked immunosorbent assay. Functionally, lung involvement was assessed by pulmonary function tests and radiologically by chest radiography and high-resolution computed tomography of the lungs.

Results Mean serum IL-23 level was significantly highly elevated in SSc patients compared with healthy controls ($P<0.005$). Patients with elevated IL-23 levels exhibited shorter disease duration ($P<0.05$). Moreover, mean serum IL-23 level was elevated in diffuse SSc cases compared with limited SSc cases and in cases with pulmonary fibrosis ($P<0.05$), although they were not associated with other clinical features. Elevated mean serum IL-23 level was significantly higher in mild restrictive cases compared with moderate and severe restrictive cases. As regards high-resolution computed tomography, mean serum IL-23 level was statistically highly significantly elevated in cases with ground-glass appearance ($P<0.001$) compared with others.

Conclusion Alterations in serum concentrations of IL-23 support the hypothesis that IL-23 is associated with induction of SSc generally and SSc associated with interstitial lung disease specifically. Presumably, blockage of IL-23 could be used as a potential therapeutic target in early SSc.

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Introduction

Systemic sclerosis (SSc) is a heterogeneous chronic, autoimmune, multisystem connective tissue disorder characterized by vasculopathy, inflammation, and progressive fibrosis of the skin and internal organs [1]. Interstitial lung disease (ILD) is a major common complication along with pulmonary arterial hypertension, which is the leading cause of morbidity and mortality in scleroderma patients [2]. The diagnosis of ILD hinges on careful clinical evaluation together with pulmonary function tests (PFTs) and high-resolution computed tomography (HRCT) [3].

Interleukin 23 (IL-23) is a heterodynamic cytokine belonging to the IL-6/IL-12 family, which can have both proinflammatory and anti-inflammatory effects on the development of autoimmune pathology. This family mediates distinct roles and cellular functions, coordinating regulation of Th1 development and type 1 cell-mediated immunity [4].

The pathogenesis of the disease is still obscure, but strong evidence suggests an immune dysregulation in the development of SSc. $CD8^+$ T cells can be found dominantly in lungs, and $CD4^+$ T cells have cytokine profiles characterizing Th2, such as IL-4, IL-10, IL-13, IL-17, and IL-23 [5].

Another T-cell subtype is Th17, which is different from Th1 and Th2 that are involved in the development of many autoimmune diseases. IL-23 activates Th17 cells with subsequent secretion of IL-17 [6]. The IL-23–IL-17 axis is a central player in the development of chronic inflammation and in the host defense against extracellular pathogens [7].

Aim

This study aimed to assess the relationship between serum level of IL-23 and ILD in SSc.

Patients and methods

Patients

In all, 30 SSc patients, diagnosed according to the American Colleague of Rheumatology (1980) criteria

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[8], together with thirty age-matched and sex-matched apparently healthy volunteers, were enrolled in this study as a control group. All patients gave their formal consent, The protocol was approved the Ethical committee of the faculty.

These patients were selected from those attending the inpatient and the outpatient clinics of the Rheumatology and Rehabilitation and Chest Departments of Benha University Hospitals between June 2014 and January 2016.

Inclusion criteria

Patients who fulfilled the American Colleague of Rheumatology (1980) criteria were included in this study [8].

Exclusion criteria

Exclusion criteria were as follows: patients who were smokers, known to be diabetics, those with a history of acute or chronic liver or kidney diseases, those who had a recent history of infection or other inflammatory or autoimmune diseases, those receiving cytotoxic or other immunosuppressive therapy, and patients with other causes of ILD.

All SSc patients were subjected to the following. Complete patient history was taken through clinical examination with complete respiratory system examination. Skin score was measured using the technique of the modified Rodnan total skin thickness score [9]. Laboratory investigations included complete blood count, erythrocyte sedimentation rate, and C-reactive protein; antinuclear antibody (ANA) was determined by indirect immunofluorescence; antiscleroderma 70 (anti-Scl 70) antibody and anticentromere antibody were detected by enzyme-linked immunosorbent assay (ELISA); and serum IL-23 was detected by human IL-23 ELISA kits (R&D Systems, Minneapolis, Minnesota, USA) [10]. About 2 ml of venous blood was collected by sterile venipuncture, allowed to clot, and sera were separated and kept frozen at − 20°C until they were used for the detection of serum IL-23 level. All samples were analyzed using the average of the optical density values to calculate concentrations. The minimum detection limit (cutoff) was calculated as the average value of the blanks plus 2 SD. The reaction is terminated by the addition of acid, and absorbance is measured at 450 nm.

Lung involvement was assessed functionally by PFT spirometry, forced expiratory volume in the 1 s (FEV_1%), forced vital capacity% (FVC%), and FEV_1/FVC. PFTs were done using sensor medics Spirolab III (Rome, Italy) according to American thoracic society, radiologically by chest radiograph posteroanterior view, and HRCT of both lungs as explained by Goh *et al.* [11]. The computed tomography scans were performed with a scanner (Prospeed S Scaner, GE YMS Ltd, Tokyo, Japan) at 10-mm intervals from the apex of the lung to the diaphragm using 1–2 mm collimator with the HRCT technique. All patients underwent scanning in the supine position at full inspiration. No intravenous contrast agent was used. Images were obtained at a window width of 750 HU and a window level of −800 HU. Echocardiography was done to evaluate pulmonary artery pressure and to assess whether there was secondary pulmonary hypertension.

Statistical analysis

Data were tabulated and statistically analyzed using statistical package for the social science (SPSS, version 16; SPSS INC., Chicago, Illinois, USA) [12].

Qualitative data were represented as frequencies and relative percentages. $\chi 2$-test was used to calculate the difference between qualitative variables.

Quantitative data were expressed as mean±SD. Independent *t* test was used to calculate the difference between quantitative variables in two groups. Mann–Whitney test was used to calculate the difference between quantitative variables in two groups in non-normally distributed data.

Analysis of variance *F*-test test was used to calculate the difference between quantitative variables in more than two groups. Kruskal–Wallis *K* test was used to calculate the difference between quantitative variables in more than two groups in non-normally distributed data. Pearson's correlation coefficient used to calculate the correlation between quantitative variables.

The significance level for all the above-mentioned statistical tests was calculated. The threshold of significance is fixed at 5% level (*P*-value): *P* value greater than0.05 indicates nonsignificant results, *P* value less than 0.05 indicates significant results, and *P* value less than 0.001 indicates highly significant results.

Results

This study included 30 SSc patients and 30 age-matched and sex-matched apparently healthy controls. Nine (30%) SSc patients had limited SSc (LSSc) and 21 (70%) had diffuse SSc (dSSc). Characteristics of the studied groups are shown in

Table 1. There were no statistically significant differences between cases and controls as regards age and sex distribution ($P>0.05$). As regards mean serum IL-23 levels, there was a highly statistically significant difference – being higher in SSc patients. All differential leukocytic counts were within normal range, and no eosinophilia was detected.

Table 2 showed the relation between mean serum IL-23 level and the studied variables in SSc patients.

There was NO statistically significant difference as regards patients sex, statistically highly significant difference being higher in dSSc patients compared to LSSc patients ($P=0.005$). Mean serum IL-23 level was higher in cases with shorter disease duration (≤5 years) ($P=0.02$), with a statistically significant difference compared with patients with longer disease duration.

About 100% of SSc patients had cutaneous manifestations in the form of Raynaud's disease, hypopigmentation and hyperpigmentations, digital ulcers, calcinosis, and telangiectasia; 23.3% had arthritis; 80% had gastroesophageal reflux; 80% had cough; 76.7% had dyspnea; 16.7% had pulmonary hypertension; and 6.7% had sicca manifestations, which classically combine dry eyes and dry mouth.

Mean serum IL-23 level was significantly higher in SSc patients with cardiopulmonary manifestation in the form of cough, dyspnea, and chest pain ($P<0.001$), with no statistically significant difference as regards PHT, skin manifestations, musculoskeletal manifestations, gastrointestinal manifestations, and sicca symptoms (dryness of the eyes and mouth) ($P>0.05$).

In this study, mean serum IL-23 levels were significantly higher in ANA and anti-Scl-70 positive patients as compared with others ($P<0.05$).

As regards radiological findings of SSc patients, there was a statistically significant difference ($P=0.01$) as regards mean serum IL-23 level in patients with pulmonary fibrosis by chest radiography, and there was a highly statistically significant difference in cases with reticular and ground-glass appearance ($P<0.001$).

Table 3 shows PFTs of the two studied groups.

FVC% and FEV_1% were significantly higher in the control group than in cases, with no statistically significant difference regarding FVC%/FEV_1%.

Figure 1 shows that SSc cases with interstitial fibrosis pattern in chest radiography have significantly higher mean serum levels of IL-23 than cases with normal chest radiography, and by HRCT; cases with ground-glass pattern and reticular pattern have significantly higher mean serum IL-23 levels than cases with normal, reticulonodular, and honeycomb patterns.

Figure 2 shows that mean Serum IL-23 levels were significantly higher in mild restrictive cases compared with moderate and severe cases, and also higher in moderate restrictive cases compared with severe restrictive cases.

Discussion

SSc is a chronic, multisystem connective tissue disease of unknown etiology. IL-23 is a member of the fascinating family of cytokines, which can have both proinflammatory and anti-inflammatory effects on the development of autoimmune pathology [4].

This work showed that there was a highly statistically significant difference as regards mean serum IL-23 level in the studied groups, being higher in SSc

Table 1 Characteristics of the studied groups

Variables	Cases (n=30)	Control (n=30)	Tests	P-value
Age (years) (mean±SD)	39.6±6.54	36.2±7.5	1.87 (t)	0.07 (NS)
Sex (male/female)	9/21 (30/70)	7/23 (23.3/76.7)	0.34 (χ^2)	0.56 (NS)
Disease duration (years) (mean±SD)	6.7±3.05	–	–	–
Modified Rodnan score (mean±SD)	21.3±7.04	–	–	–
ESR (mm/1st h) (mean±SD)	44.27± 33.05	–	–	–
CRP (mg/dl) (mean±SD)	5.34±2.95	–	–	–
HB (g/dl) (mean±SD)	9.45±0.71	–	–	–
WBCs ($\times10^3$/mm^3) (mean±SD)	6.05±1.72	–	–	–
Platelets ($\times10^3$/mm^3) (mean±SD)	266.23 81.33	–	–	–
IL-23 (pg/ml)				
Mean±SD	75.78±34.13	26.72±5.84	4.84	<0.001
Range	23–122	17.65–32.8		

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HB, hemoglobin; IL-23, interleukin-23; WBCs, white blood cells.

Table 2 Comparison between mean serum interleukin-23 levels and the studied variables in systemic sclerosis patients

Variables	IL-23			MW	*P*-value
	N	Mean	SD		
Sex					
Male	9	86.18	23.23	0.93	0.37 (NS)
Female	21	71.33	37.23		
Classification					
LSSc	9	46.87	38.99	2.72	0.005
dSSc	21	88.17	23.31		
Duration					
≤5 years	12	93.2	41.67	2.29	0.02
>5 years	18	64.17	22.49		
Clinical manifestations					
Skin manifestations					
Raynaud's disease					
No	33.9	64.34	3	0.76	0.47 (NS)
Yes	34.6	77.05	27		
Finger-tip lesions					
No	32.4	72.46	15	0.71	0.48 (NS)
Yes	37.1	80.12	15		
SC calcinosis					
No	32.6	75.55	24	0.10	0.94 (NS)
Yes	43.3	76.71	6		
Telangiectasia					
No	32.4	82.15	21	1.31	0.19 (NS)
Yes	35.2	70.93	9		
MSC manifestations					
Tendon flexion rub					
No	25	79.12	34.1	1.19	0.25 (NS)
Yes	5	60.08	32.4		
Arthritis					
No	23	83.94	33.1	1.57	0.08 (NS)
Yes	7	68.98	22.9		
Myositis					
No	25	77.95	30.8	0.39	0.71 (NS)
Yes	5	64.94	50.6		
GIT manifestations					
No	6	75.77	33.1	0.13	0.90 (NS)
Yes	24	75.79	35.1		
Pulmonary manifestations					
Pulmonary HPT					
No	25	64.55	4.8	2.03	0.07 (NS)
Yes	5	79.81	38.2		
Cough					
No	6	27.66	3.1	3.5	<0.001
Yes	24	87.81	26.7		
Dyspnea					
No	7	27.31	2.9	3.95	<0.001
Yes	23	90.54	23.6		
Chest pain					
No	10	45.69	29.7	3.08	<0.001
Yes	20	90.83	25.4		
Other					
Sicca (dryness) symptoms					
Yes	28	79.39	32.4	1.16	0.31 (NS)
No	2	75.21	2.7		
Immunological					
ANA					
Negative	2	24.11	1.56	2.29	0.005

(*Continued*)

Table 2 (*Continued*)

Variables	IL-23			MW	*P*-value
	N	Mean	SD		
Positive	28	79.47	32.23		
Anti-Scl 70					
Negative	11	46.68	38.55	2.79	0.004
Positive	19	88.25	23.45		
Anti-centromere					
Negative	22	83.87	25.65	1.70	0.09 (NS)
Positive	8	56.90	44.77		
Radiological					
Radiography					
Normal	13	61.48	46.05	2.35	0.01
Interstitial fibrosis	17	79.54	19.54		
HRCT					
Normal	7	35.31	2.9		
Mixed reticular and ground-glass appearance	13	116.11	4.79	27.17 (*F* test)	<0.001
Reticulonodular and honeycomb appearance	10	75.63	7.05		

ANA, antinuclear antibodies; anti-SCl-70, anti-scleroderma-70; GIT, gastrointestinal; HPT, hypertension; HRCT, high-resolution computed tomography; MSC, musculoskeletal; MW, Mann–Whitney test.

Table 3 Pulmonary function tests of the two studied groups

Variables	Cases (*n*=30)	Controls (*n*=30)	*t* Test	*P*-value
FVC%				
Mean±SD	61.33±20.8	85.57±8.43	5.19	<0.001
Range	18–85	75–106		
FEV_1%				
Mean±SD	66.8±19.04	87.93±7.35	5.67	<0.001
Range	23–96	76–100		
FEV_1/FVC				
Mean±SD	109.97±10.13	109.8±13.58	0.05	0.96
Range	91–126	85–125		
Severity [*n* (%)]				
Normal	7 (23.3)	30 (100)	32.3 (χ^2)	<0.001
Mild restrictive	13 (43.3)	0 (0)		
Moderate and severe restrictive	10 (33.3)	0 (0)		

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

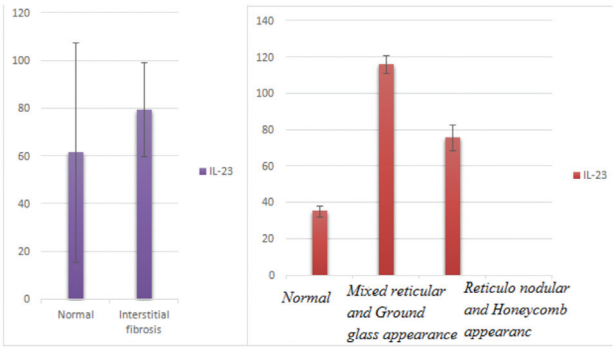
patients. These results were in agreement with those of Murata *et al.* [13], who reported higher concentration of IL-23 in SSc than in healthy controls; as IL-23 production appears to be of great importance in the inflammatory reaction of SSc, it causes the activation of Th17 cell, producing IL-17, a proinflammatory cytokine that induces the production of other proinflammatory cytokines. In contrast, Mathian *et al.* [14] and Olewicz *et al.* [15] found decreased serum levels of IL-17 and IL-23 in SSc patients compared with healthy patients. These different results can result from differences in the treatment or differences in disease duration in the studied group or may be related to small sample size of SSc.

Moreover, Agarwal *et al.* [16] reported that the gene encoding for IL-23 receptor has been identified as a susceptibility gene for SSc development, and IL-23R polymorphisms are associated with antitopoisomerase-I positivity and lower frequency of pulmonary hypertension, which further support the increased mean serum IL-23 level in SSc patients.

In this study, mean serum IL-23 level was found to be statistically significantly elevated in dSSc patients compared with LSSc patients (P=0.005). In contrast to our results, Komura *et al.* [17] and Olewicz *et al.* [15] showed no statistically significant difference between mean serum IL-23 levels among dSSc and LSSc groups.

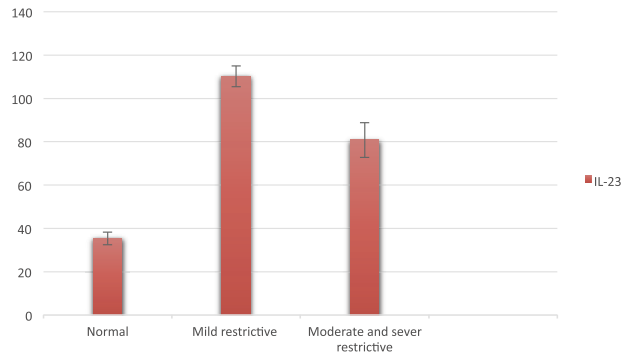
In this study, there was a statistically significant difference in cases with shorter disease duration (≤5 years) (P=0.02) than cases with longer disease duration. In agreement with our results, Komura *et al.* [17] and Olewicz *et al.* [15] found that SSc patients with disease duration less than 2 years had significantly elevated IL-23 level compared with those with

Figure 1



Relation between mean serum interleukin 23 (IL-23) levels and radiological finding of the systemic sclerosis patients.

Figure 2



Relation between mean serum interleukin 23 (IL-23) levels and grading of pulmonary function tests of the systemic sclerosis patients.

duration of 2–5 years ($P<0.05$) and those with duration more than 5 years ($P<0.01$). This may be explained by decreased activity of Th17 cells along with disease progression and immunosuppressive drugs [15]. Thus, the level of IL-17 was noted to be higher early in SSc patients, with subsequent progressive reduction of IL-17 levels along with disease duration. Moreover, IL-23 is known as a survival and proliferative factor for Th17 cells [18]. Some reports recommend that not all Th17 cells are pathogenic, and exposure to antigen-presenting-cell-derived IL-23 is crucial for their ability to stimulate autoimmunity and inflammation [19].

In this study, mean serum IL-23 level was significantly elevated in SSc patients with cardiopulmonary manifestation in the form of cough, dyspnea, and chest pain) ($P<0.001$), with no statistically significant difference as regards PHT and other clinical manifestations ($P>0.05$). Similarly, Komura *et al.* [17] have described that elevated mean IL-23 level was related to the presence of pulmonary fibrosis, but not to other clinical features of SSc. This may be related to an increased number of Th17 cells with enhanced skin-homing and lung-homing properties in SSc individuals [20]. In contrast, Radstake *et al.* [21] and Demir *et al.* [22] showed no statistically significant difference between mean serum IL-23 level and any clinical features of SSc.

In this study, as regards immunological profile, mean serum IL-23 level was significantly elevated in ANA-positive and anti-Scl-70-positive patients compared with others ($P<0.05$). Similarly, Gourh *et al.* [23] reported that IL-23 levels were more elevated in the anti-Scl-70 antibody and ANA-positive group. On the other hand, Demir *et al.* [22] found that there was no correlation between serum IL-23 levels and ANA, anti-Scl-70, and anti-centromere antibody positivity.

In this study, SSc cases with interstitial fibrosis pattern by chest radiography had significantly higher mean serum levels of IL-23 than cases with normal chest radiography. By HRCT, cases with mixed reticular and ground-glass patterns compared with reticulonodular and honeycomb patterns have significantly higher mean serum IL-23 levels than group of normal. By PFT, mean serum IL-23 levels were significantly higher in mild restrictive cases than in moderate and severe cases.

Komura *et al.* [17] and Olewicz *et al.* [15] found a significant correlation between serum IL-17and IL-23 concentration and the extent of lung damage in SSc patients as evaluated with HRCT. In contrast to these results, there was no difference between the frequency of Th17 bronchoalveolar lavage T cells in SSc patients with interstitial lung fibrosis, SSc patients without ILD, and healthy controls [24].

The study by Kurasawa *et al.* [25] revealed that IL-17 messenger RNA was expressed in unstimulated lymphocytes from the peripheral blood and lymphocytes from the skin and lungs of SSc patients, but not from patients with systemic lupus erythematosus or polymyositis/dermatomyositis or from healthy donors. IL-17 levels were also increased in the serum of SSc patients, but not in that of systemic lupus erythematosus patients or healthy donors. IL-17 overproduction was significantly related to the early stage of SSc, but not to other clinical features of SSc. Moreover, IL-17 enhanced the proliferation of fibroblasts and induced the expression of adhesion molecules and IL-1 production in endothelial cells *in vitro* and concluded that IL-17 is overproduced by T cells from the peripheral blood and fibrotic lesions of the skin and lungs in SSc patients. These results suggest that IL-17 overproduction has an important role in the pathogenesis of SSc,

especially in the early stages of the disease, by inducing the proliferation of fibroblasts and the production of IL-1 and the expression of adhesion molecules on endothelial cells.

Conclusion

Alterations in serum concentrations of IL-23 support the hypothesis that IL-23 is associated with induction of SSc generally and SSc associated with ILD

Specifically and that blockage of IL-23 could be used as a potential therapeutic target in early SSc.

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Conflicts of interest

There are no conflict of interest.

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