Interleukin-6 in systemic sclerosis and potential correlation with pulmonary involvement

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Abstract  Background: A progressive pulmonary involvement is frequent in systemic sclerosis and it is the leading cause of morbidity and mortality. IL-6 has been implicated in the pathogenesis of systemic sclerosis via stimulation of fibroblasts to produce excess collagen and glycosaminoglycan. Specific correlation between IL-6 and lung involvement have not been found yet.

Aim: To study the possible correlation between lung involvement (assessed by spirometry and HRCT abnormalities) and the serum level of IL-6.

Subjects and methods: 20 patients with scleroderma compared with 20 matched volunteers as control group. All participants underwent spirometry, HRCT scan and serum IL-6 measurements. HRCT signs were scored according to Warrick et al. score for systemic sclerosis.

Results: Patients showed a statistically significant reduction in FVC%, FEF 25–75% and significantly higher ESR and IL-6 compared to control. There was a highly significant positive correlation between the total HRCT score and serum IL-6.

Conclusion: Serum IL-6 could be a marker of the degree of pulmonary involvement in patients with systemic sclerosis.

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Introduction

Systemic sclerosis (SS) is a diffuse connective tissue disease of unknown etiology, characterized by skin and visceral fibrosis, vascular dysfunction, and circulating autoantibodies [1]. Pulmonary involvement is frequent in SS. It is reported in 70% [2] to 100% [3] of patients in autopsy series.

Several cytokines and chemokines have been implicated in the induction of fibrosis, but a definitive relationship between specific cytokines and some organ involvement has not been
established yet [4]. IL-6, one of the pro-inflammatory cytokines, has been implicated in the pathogenesis of SS. IL-6 expression is reportedly high in both the skin and serum of SS patients [5], and its elevation depends on the skin score [6].

Open lung biopsy remains the gold standard to diagnose interstitial lung disease, high resolution CT (HRCT) is considered to be the reference among the noninvasive procedures. It allows very precise analysis of lung parenchyma, with the advantage of assessing lung volumes [7]. Interstitial signs on HRCT are not equal in significance. Honeycombing, for example, has a worse prognosis than ground-glass appearance [8]. Warrick et al. [9] have defined a score based on the criteria of type and extent of HRCT signs in SS patients. The aim of this study is to study the possible correlation between lung involvement (assessed by spirometry and HRCT abnormalities) and the serum level of IL-6 in SS patients.

Patients and methods

Twenty patients (6 males and 14 females) with SS were recruited from the rheumatology outpatient clinic in Minoufiya University Hospital during the period from Oct 2012 to Oct 2013 and 20 age and sex- matched apparently healthy control subjects. All patients fulfilled the criteria proposed by the American College of Rheumatology [10]. None of the patients had recent symptoms or signs of lower respiratory infection at the time of the study. Known histories of asthma, allergic alveolitis, or exposure to organic dusts were exclusion criteria. Informed consent and ethical approval from Minoufiya University Hospital Ethics Committee were obtained from all participants before enrollment.

Pulmonary function test

Pulmonary function tests (PFT) were done for all the patients in the pulmonary function test unit in Minoufiya University Hospital using spirometer (Quark PFT3, COSMED, Italy). All measurements were performed according to the American Thoracic Society recommendations and expressed as percent of predicted values based on age, sex and height. Clinically significant restrictive lung disease was defined when an abnormal FVC with normal FEV1/FVC was observed [11].

HRCT scanning

All participants underwent a HRCT scan of the chest. HRCT scans were read by two independent radiologists in random order, without knowledge of the results of the other’s findings. A consensus was obtained in all cases after a common third read. Interstitial signs on HRCT were not equal in significance. Honeycombing, for example, has a worse prognosis than ground-glass appearance [8]. Warrick et al. [9] have defined a score based on the criteria of type and extent of HRCT signs in SS patients. The aim of this study is to study the possible correlation between lung involvement (assessed by spirometry and HRCT abnormalities) and the serum level of IL-6 in SS patients.

IL-6

Serum IL-6 levels were examined by ELISA, as described by manufacturer (Biosource, Nivelles, Belgium); fasting venous blood samples were taken from all participants when HRCT or PFTs were carried out.

Statistical analysis

Data were analyzed by the SPSS version 13.0 statistical package. Categorical and quantitative variables were respectively described as numbers, percentage (%) and mean ± standard deviation (SD). Between-group comparisons were performed using Student’s t test for variables with a normal distribution and the Mann–Whitney U-test for variables with a non-normal distribution. Correlation between variables was calculated by Pearson correlation coefficient. P values < 0.05 were considered statistically significant.

Results

This study was conducted on 20 patients with SS (6 males and 14 females) and another 20 control subjects (8 males and 12 females). The FVC% and FEF 25–75% were significantly lower in the patients compared to controls. There was no significant difference between the two groups regarding age, sex and FEV1/FVC (Table 1).

There were significantly higher values of ESR, CRP, Anti Scl-70 and IL-6 in patients compared to controls Table 2.

In the studied patients, there was a significantly positive correlation between serum IL-6 level and each of CRP, Anti Scl-70 and HRCT score. Table 3, Figs. 1–3.

Discussion

Several lines of evidences indicate that SS presents deregulated production of cytokines implicated in vascular damage and fibrosis, but their relationship with clinical findings is still unclear. We studied the possible correlation between lung involvement (assessed by PFT and HRCT abnormalities) and the serum level of IL-6. We found significantly higher levels of serum IL-6 in patients with SS compared to controls (Table 2). Moreover, serum IL-6 correlated positively with Anti Scl-70 (Table 3, Fig. 3). This goes in agreement with several studies [5,12–14]. IL-6 is a pleiotropic cytokine with multiple biological effects on immune regulation, haematopoiesis, inflammation, oncogenesis [15–20]. Several authors [12,13,21] have demonstrated increased production of IL-6 by fibroblasts in SS patients. Duncan et al. [22] study showed furthermore increased production of collagen and glycosaminoglycans, hyaluronic acid and chondroitin-4/6-sulfates from human dermal fibroblasts induced by IL-6 suggesting a role for IL-6 in promoting fibrosis, and increased levels of IL-6 have been reported in the serum, bronchoalveolar lavage, and skin biopsies of patients with SS [5,18,21,23]. Moreover, Lung fibrosis induced by irradiation or bleomycin therapy is attenuated in IL-6 gene knockout mice [24].

The present study demonstrated a highly significant positive correlation between serum IL-6 and CRP (Table 3, Fig. 2). This is in agreement with Alegre-Sancho et al. [25]
and Ohtsuka et al. [26] studies in which IL-6 level showed a moderate correlation with high-sensitivity CRP level. CRP level is a general marker of inflammation. It is a part of the innate immune response to systemic inflammation [27]. IL-6 and CRP have related roles in the inflammatory response: IL-6 induces CRP production in the liver by activating Janus kinases. Signal transducers and activators of transcription subsequently switch on the CRP gene expression, leading to the production of CRP [28]. CRP and/or ESR have been linked to lung involvement in SS [29].

In the current study, there was a highly significant positive correlation between serum IL-6 and HRCT score (Table 3, Fig. 1). This matches the results of De Santis et al. [30] study. Although they used a different HRCT scoring system as described by Kazerooni et al. [31] They demonstrated a significantly positive correlation between HRCT score and IL-6 plasma level \( r = 0.36, P = 0.0012 \) suggesting that patients with a greater extent of lung affection on HRCT had a more aggressive disease. In addition, higher IL-6 plasma levels suggest that inflammation could explain the more aggressive pulmonary disease in patients with alveolitis. Our results also agree with Diot et al. [32] study who studied the abnormalities on lung HRCT in 52 patients with SS using the Warrick et al. [9] score and with reference to the PFT results of each patient. They found that HRCT score was significantly higher in

### Table 1  Demographic and Spirometric data of the studied groups.

<table>
<thead>
<tr>
<th>The studied groups</th>
<th>Test of significance</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases No = 20</td>
<td>Controls No = 20</td>
<td>( t )-test = 0.02</td>
</tr>
<tr>
<td>Age (years) Mean ± SD</td>
<td>34.60 ± 11.82</td>
<td>34.70 ± 11.12</td>
</tr>
<tr>
<td>Male (N, %)</td>
<td>6 (30%)</td>
<td>8 (60%)</td>
</tr>
<tr>
<td>FVC% (Mean ± SD)</td>
<td>64.60 ± 26.45</td>
<td>88.35 ± 5.37</td>
</tr>
<tr>
<td>FEV1/FVC (Mean ± SD)</td>
<td>87.80 ± 6.46</td>
<td>87.20 ± 4.87</td>
</tr>
<tr>
<td>FEF 25–75% (Mean ± SD)</td>
<td>58.80 ± 16.65</td>
<td>80.90 ± 8.19</td>
</tr>
</tbody>
</table>

FVC: forced vital capacity, FEV1: forced expiratory flow in the first second, FEF 25–75%: forced expiratory flow 25–75%.

* Statistically significant \( (P < 0.05) \).

### Table 2  Laboratory values of the studied groups.

<table>
<thead>
<tr>
<th>The studied groups</th>
<th>( t )-test</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases No = 20</td>
<td>Controls No = 20</td>
<td>( t )-test = 5.2</td>
</tr>
<tr>
<td>ESR mm (Mean ± SD)</td>
<td>26.7 ± 16.96</td>
<td>5.55 ± 2.31</td>
</tr>
<tr>
<td>CRP mg/L (Mean ± SD)</td>
<td>18.5 ± 13.79</td>
<td>3.72 ± 1.65</td>
</tr>
<tr>
<td>Anti Scl-70 U/ml (Mean ± SD)</td>
<td>32 ± 7.49</td>
<td>8.55 ± 2.79</td>
</tr>
<tr>
<td>IL-6 pg/ml (Mean ± SD)</td>
<td>5.55 ± 3.92</td>
<td>1.49 ± 1.87</td>
</tr>
</tbody>
</table>

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Anti Scl-70; anti scleroderma-70.

** Statistically highly significant \( (P < 0.001) \).

### Table 3  Correlation between IL-6 and other parameters in the studied patients.

<table>
<thead>
<tr>
<th>IL-6</th>
<th>( r )</th>
<th>( P )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.40</td>
<td>0.078</td>
</tr>
<tr>
<td>ESR</td>
<td>−0.19</td>
<td>0.93</td>
</tr>
<tr>
<td>CRP</td>
<td>0.91</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Anti Scl-70</td>
<td>0.90</td>
<td>0.000 **</td>
</tr>
<tr>
<td>FVC%</td>
<td>0.31</td>
<td>0.19</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>0.64</td>
<td>0.20</td>
</tr>
<tr>
<td>FEF 25–75%</td>
<td>0.29</td>
<td>0.22</td>
</tr>
<tr>
<td>Chest HRCT score</td>
<td>0.97</td>
<td>0.000 **</td>
</tr>
</tbody>
</table>

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Anti Scl-70; anti scleroderma-70, FVC: forced vital capacity, FEV1: forced expiratory flow in the first second, FEF 25–75%: forced expiratory flow 25–75%, HRCT: High resolution CT.

* Statistically significant \( (P < 0.05) \).

** Statistically highly significant \( (P < 0.001) \).

**Figure 1** Correlation between IL-6 and HRCT score in the studied patients.
patients with diffuse SS than in those with limited SS. Moreover, HRCT score negatively correlated with diffusing capacity (DLCO), total lung capacity (TLC) but not FVC.

There was no correlation between IL-6 and FVC, FEV1/FVC nor FEF 25–75% (Table 3). These parameters are usually affected later than declined DLCO which is the early PFT abnormality encountered in patients with interstitial lung disease. De Santis et al. [30] study showed that serum IL-6 was associated with DLCO decline but not FVC in SS, and therefore, they considered it a good biomarker of PFT affection candidate to be investigated further. The analysis of their test cohort showed that elevated IL-6 was predictive of a decline in FVC and/or DLCO within the first year, and of death within the first 30 months. They explained the lack of association with progression at later time points by the fact that IL-6 might signal the onset of phases of disease progression, rather than mark different phenotypes of the disease constitutively characterized by a different prognosis. In conclusion; Serum IL-6 measurement may be a noninvasive independent predictor of lung involvement and progression in SS patients. Prospective studies are needed to evaluate the exact role of IL-6 in lung fibrosis, to assess whether there could be a role for anti-IL-6 treatment in SS interstitial lung disease.

A number of limitations should be considered when interpreting our findings. There were no data regarding the duration of SS disease prior to study enrollment, no differentiation of patients weather they have diffuse or limited SS, No data about SS therapy, Pulmonary function tests did not include DLCO.

Conflict of interest

None declared.

References


Figure 2 Correlation between IL-6 and CRP in the studied patients.

Figure 3 Correlation between IL-6 and anti Scl-70 in the studied patients.
Systemic sclerosis and lung involvement