Circulating adipokines and organ involvement in patients with systemic sclerosis

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ABSTRACT

Background: In recent years, mediators synthesized in the adipose tissue, the so-called adipokines, have been reported to play important roles in the pathogenesis of autoimmune rheumatic diseases.
Objective: To compare serum leptin, adiponectin and resistin levels in patients with systemic sclerosis (SSc) and healthy controls. To find possible relationship between serum levels of adipokines and organ involvement with focus on interstitial lung disease in SSc patients.
Patients and Methods: Lung involvement was assessed functionally (body plethysmography, diffusing capacity of the lung for carbon monoxide (DLCO) and six-minute walk test) and radiologically (using average disease extent on high resolution computed tomography (HRCT) of the lungs, according to the percentage of interstitial changes) in 29 SSc patients. Quantitative sandwich ELISA was used to measure resistin, leptin and adiponectin concentrations in sera of patients and 30 healthy controls.
Results: We found no statistically significant differences in serum resistin, leptin and adiponectin levels between SSc patients and the controls. However, serum adiponectin concentrations were significantly lower in active than in inactive patients. They also correlated positively with vital capacity (VC) (p=0.04) and negatively with Valentini disease activity index (p=0.04). Serum resistin levels were significantly elevated in patients with digital ulcers (p=0.03) and serum concentrations of leptin were associated with the duration of SSc symptoms other than Raynaud's phenomenon (p<0.01)
Conclusions: Serum adiponectin should be further investigated as a candidate for SSc activity marker and resistin may play a role in ulcer development in SSc patients.

Keywords: Scleroderma; Adipokines; Pulmonary fibrosis.

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INTRODUCTION

Systemic sclerosis (SSc) is a chronic, complex disease of unknown etiology characterized by excessive fibrosis, vascular damage and inflammation[1]. A leading cause of morbidity and mortality in SSc patients is lung involvement[2] presented as two major pulmonary syndromes: pulmonary arterial hypertension (PAH) and interstitial lung disease (ILD). White adipose tissue is known to be an active organ that secretes proteins called adipokines. Adipokines take part in regulation of many pathophysiological processes, both metabolic and inflammatory, but their significance remains obscure[3].

Resistin is a 12.5 kDa polypeptide which in humans is highly expressed in bone marrow and lung[4] and is secreted mainly by immune cells[5]. It is a cysteine-rich protein which is robustly induced in response to various proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin (IL)-6, IL-1β, and resistin itself, and has been shown to up-regulate the expression of proinflammatory cytokines such as TNF-α, IL-6 and IL-12[6-8]. The co-culture of T cells and dendritic cells treated with resistin enhances the expansion of regulatory T cells, an important source of transforming growth factor β (TGF-β), a key profibrotic cytokine[9].

Adiponectin is a 30 kDa plasma protein mainly produced in white adipose tissue by mature adipocytes and non-fat cells, although it can also be found in skeletal muscle cells, cardiac myocytes and endothelial cells[10]. Its secretion is suppressed by TNF-α and its production is also regulated by other proinflammatory cytokines, like IL-6[11].

The third cytokine-like hormone, leptin, is a circulating non-glycosylated peptide of 16 kDa[12]. Leptin exerts effect on the different cell populations of both innate and adaptive immune responses. It up-regulates the secretion of proinflammatory cytokines like TNF-α, IL-6, IL-12, increases the expression of adhesion molecules, induces the expression of activation markers of monocytes, NK cells and lymphocytes[13]. Leptin and adiponectin, and their respective receptors are also expressed in the human lung[14,15].

A growing body of evidence indicates that these three adipokines play important roles in autoimmune diseases. Resistin is associated with disease activity and laboratory findings of systemic lupus erythematosus (SLE)[16], rheumatoid arthritis (RA)[17] and psoriatic arthritis[18], and it was also described as a useful marker of pulmonary vascular involvement in SSc[19]. In patients with diffuse SSc, tissue levels of adiponectin have been shown to inversely correlate with skin scores[20]. The study by Fang et al. demonstrated the anti-fibrotic effects of adiponectin in normal and scleroderma fibroblasts[21]. Leptin was found to have opposing, profibrotic effects, as it promotes the development of bleomycin-induced lung fibrosis in mice by augmentation of TGF-β signaling[22]. As literature data on serum adipocytokines levels in SSc patients are sparse and controversial, this study aims to investigate the relationship and possible interactions between resistin, leptin, adiponectin and markers of disease activity and organ involvement in patients with SSc, with particular regard to lung involvement.

MATERIAL AND METHODS

PATIENTS

We recruited to the pilot study 29 consecutive Caucasian patients with SSc, according to the American College of Rheumatology (ACR) classification criteria[23]. All patients were also analysed using the 2013 ACR/EULAR classification criteria for SSc[24] and their age was greater than 18 years. Patients with mixed connective tissue disease or overlap syndrome were excluded from the study. Moreover, all the patients were grouped according to the 2-cutaneous subset classification[25] as having diffuse cutaneous (dcSSc, cutaneous thickening of both distal and proximal extremities, with or without truncal involvement) or limited cutaneous (lcSSc, cutaneous thickening of both extremities distally to the elbows and knees, with or without sclerosis of the neck and face) form of the disease. A further subdivision was made into early SSc (n=13) and late SSc (n=16) based upon the duration of the disease, defining early SSc as patients having the disease duration of less than 2 years and late SSc for those longer than 2 years. Healthy controls (n=30, 27 females, 3 males) were volunteers and were statistically matched for gender and age (18-29 years, 30-44 years, 45-54 years, 55-70 years). Informed consent was obtained from all participants and the study was approved by the Institutional Review Board at Poznan University of Medical Sciences. A protocol of the conducted research conforms to the principles of the World Medical Association's Declaration of Helsinki.

CLINICAL EVALUATION AND LABORATORY MEASUREMENTS

The workup included patients' history and a thorough clinical examination which included ulcer assessment and evaluation of skin involvement using the modified Rodnan skin thickness score[26]. Body mass index (BMI) was calculated for all participants. The disease duration was measured from the onset of the first symptom, other than Raynaud's phenomenon, consistent with SSc. The disease activity of SSc was determined using European Scleroderma Study Group disease activity score for SSc (Valentini disease activity index)[27]. Myositis was evaluated as muscle weakness associated with elevated levels of serum creatine kinase. Patients with decreased glomerular filtration rate (GFR) and/or proteinuria were classified as having renal involvement. Joint involvement was defined by the detection of symmetric synovitis, flexion contractures, and tendon friction rubs. The presence of pulmonary arterial hypertension (PAH) was confirmed by right heart catheterisation. The presence of ILD was assessed with use of pulmonary function tests: body plethysmography, diffusing capacity of the lung for carbon monoxide (DLCO) and six-minute walk test, and radiologically (high resolution computed tomography of the lungs, HRCT) as proposed by Goh *et al.*[28]. All HRCT examinations were evaluated by a radiologist expert on HRCT ILD. The patients were divided into

groups based on HRCT disease extent thresholds of 5% and 20%, and HRCT scans were also assessed for the following findings: the average extent of the disease, the extent of a reticular pattern, the extent of ground glass and the presence of honeycombing. Blood samples from patients were collected at the time of clinical examination on fasting conditions. Obtained sera were stored at -70°C before the assays were performed. The degree of inflammatory activity was determined by the erythrocyte sedimentation rate (ESR, Westergren), highly sensitive C-reactive protein (CRP, enzyme-linked immunosorbent assay (ELISA), BioCheck, USA) and complement components C3 and C4 (radial immunoelectrophoresis). Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on HEp2 cells (Euroimmun, Germany) and antibodies to extractable nuclear antigens (ENA) were estimated using line immunoassay (Euroimmun, Germany). Serum levels of resistin, leptin and adiponectin were measured using commercially available ELISA kits (R&D Systems, USA) and calculated using standard curves generated with specific standards according to the manufacturer's recommendations.

STATISTICAL ANALYSIS

Patients demographic data were analysed using descriptive statistics. All contiguous data were tested for normal distribution using the Kolmogorov-Smirnov test. The number of cases was expressed as percentage, mean and standard deviation values. Other non-normally distributed parameters were compared by Mann–Whitney U-test. Correlations between variables within the group were analysed using Spearman's rank-order correlation coefficient (r). Results are expressed as medians (interquartile range, IQR) and considered statistically significant at $p<0.05$. All the analyses were performed with STATISTICA software (StatSoft, Inc (2009). STATISTICA (data analysis software system) version 9.1, www.statsoft.com).

RESULTS

Out of 29 SSc patients, 26 were female and 3 were male (median age 56 years, range 24-70). Eighteen patients had lcSSc (17 females and 1 male) and 11 had dcSSc (9 females and 2 males). All but one SSc patients had a history of Raynaud's phenomenon after exposure to low temperature, 11 (37.9%) had digital ulcers at the time of examination, 15 (51.7%) had gastrointestinal symptoms, 7 (24.1%) had hypertension, 8 (27.6%) had active arthritis, 1 (3.4%) had myositis, 1 (3.4%) had PAH and 1 (3.4%) had renal involvement. Ten patients (34.5%) showed a modified Rodnan skin thickness score of >14 and 7 out of 29 had active disease according to Valentini disease activity index. In 27 patients (93.1%) lung-function tests showed abnormal gas transfer for CO, VC was decreased in 4 (13.8%) and TLC in 1 (3.45%) out of 29 patients. Significant abnormalities on HRCT (ILD extent of > 5%) were observed in 51.7% of the subjects and ILD extent >20% was present in 24.1% of patients with SSc. There were no patients with the indeterminate extent of the disease on HRCT. Comparison between dcSSc and lcSSc revealed no statistically significant differences in pulmonary function tests with regard to percentage of predicted TLC, VC and DLCO. Further characteristics of patient group at the time of examination is shown in Table I.

We found no statistically significant differences in serum resistin, adiponectin and leptin levels either between SSc patients and healthy subjects (Table II), or between early and late SSc subjects. Likewise, there were no statistically significant differences in adipokines levels between dcSSc and lcSSc subgroups. The levels of resistin and adiponectin did not correlate with disease duration, age or sex of investigated patients.

No significant differences were found in resistin, adiponectin and leptin levels when SSc group was divided according to ILD extent of < 5% or > 5% and < 20% or >20%. But with regard to pulmonary function tests, we found statistically significant association of serum adiponectin levels with VC ($r=0.39$, $p=0.04$). We also demonstrated a significant negative correlation between adiponectin level and Valentini disease activity index ($r=-0.39$, $p=0.04$); adiponectin levels were found to be significantly lower in active patients than in inactive patients ($p=0.04$) and negatively correlated with ESR ($r=-0.48$, $p=0.01$). While resistin level manifested a significant increase in patients with digital ulcers ($p=0.03$), it showed no relationship with other investigated parameters. Serum concentrations of leptin were associated with the duration of SSc symptoms other than Raynaud's phenomenon ($r=0.67$, $p<0.01$), BMI ($r=0.53$, $p<0.01$), C3 level ($r=0.56$, $p<0.01$) and were significantly higher in smokers ($p=0.04$). However, we did not demonstrate any significant associations between median resistin, leptin and adiponectin levels and the type of treatment and other clinical status/disease activity parameters of SSc

TABLE I. CLINICAL, RADIOLOGICAL AND LABORATORY CHARACTERISTICS OF SYSTEMIC SCLEROSIS PATIENTS

Disease duration (months)		54.1 ± 68**
Raynaud's phenomenon		28 (96.6)
Digital ulcers		11 (37.9)
Gastrointestinal symptoms		15 (51.7)
Arthritis		8 (27.6)
Myositis		1 (3.4)
Pulmonary arterial hypertension		1 (3.4)
Renal involvement		1 (3.4)
Modified Rodnan skin score		13 (16)*
Antinuclear antibodies		25 (86.2)
Valentini disease activity index		2 (2.5)*
	n(%) index >3	7 (24.1)
HRCT	Extent of disease, %	15 (23.7)*
	Extent of reticular pattern, %	10 (15)*
	Extent of ground glass, %	5 (12.5)*
	Presence of honeycombing	10 (34.5)
DLCO (p%)		58.4 (16.5)*
	n(%) < 80%	27 (93.1)
Total lung capacity (p%)		108.2 (18.1)*
	n(%) < 80%	1 (3.45)
Vital capacity (p%)		98.3 (19.7)*
	n(%) < 80%	4 (13.8)
Six-minute walk test, distance (m)		375 (90)*
Erythrocyte sedimentation rate (mm/h), n(%) > 15		25 (20)*, 21 (72.4)
C-reactive protein (mg/l), n (%) > 5		0 (4.6)*, 7 (24.1)
C3 complement component (mg/l), n (%) < 900		1162 (236)*, 1 (3.4)
C4 complement component (mg/l), n (%) < 200		191 (66)*, 16 (55.2)
Treatment with cyclophosphamide		9 (31)
Treatment with methotrexate		5 (17.2)
Treatment with azathioprine		2 (6.9)
Current smokers		6 (20.7)

Data presented as the median (interquartile range)*, mean ± standard deviation** or as n (%)
HRCT, high resolution computed tomography of the lungs; DLCO, diffusing capacity of the lung for carbon monoxide

patients, including the presence of arthritis, gastrointestinal symptoms, modified Rodnan score and CRP levels. The correlations between the clinical profile of SSc patients and serum levels of the three adipokines are summarized in Table III.

DISCUSSION

The associations between adipokines and the activity and symptoms of SSc suffer from many gaps in the literature. In the present pilot study we tried to supplement the existing data in this area of research.

In this study we did not observe statistically significant differences in serum resistin level, either between SSc patients and the control or between the two SSc subtypes. This is in agreement with the previous study, where serum resistin levels were comparable between dcSSc, lcSSc and control subjects[19]. It is also in line with observations of Pehlivan *et al.*, that although patients with SSc had higher resistin levels than the control group, the difference did not reach statistical significance[29]. Moreover, other authors report that in patients with SLE resistin measurements did not differ between patients and controls[16]. On the other hand, resistin was found to be abundant in another inflamma-

TABLE II. ADIPOKINES LEVELS IN SERUM OF SYSTEMIC SCLEROSIS PATIENTS AND HEALTHY SUBJECTS

	SSc	Healthy controls
Resistin (ng/ml), median (IQR)	7.9 (2.3)	6.8 (2.4)
Leptin (ng/ml), median (IQR)	9.9 (11.6)	14.5 (22.1)
Adiponectin (µg/ml), median (IQR)	23.2 (20.2)	22.1 (10.9)

IQR, interquartile range; SSc, systemic sclerosis

TABLE III. CORRELATIONS BETWEEN SERUM LEVELS OF ADIPOKINES AND CLINICAL/LABORATORY PARAMETERS IN SYSTEMIC SCLEROSIS PATIENTS

Clinical/laboratory profile	Resistin (ng/ml)	Adiponectin (µg/ml)	Leptin (ng/ml)
Age (years)	r=0.04, p=0.84	r=0.02, p=0.92	r=0.13, p=0.51
Disease duration (years)	r=0.01, p=0.95	r=-0.08, p=0.71	r=0.67, p=0.001*
Modified Rodnan skin score	r=0.04, p=0.85	r=-0.3, p=0.12	r=-0.09, p=0.65
HRCT ILD extent (%)	r= -0.1, p=0.65	r=-0.12, p=0.58	r=0.2, p=0.38
DLCO (p%)	r= -0.1, p=0.6	r=0.31, p=0.12	r=-0.23, p=0.27
Total lung capacity p(%)	r= -0.1, p=0.6	r=0.38, p=0.05	r=-0.12, p=0.56
Vital capacity (p%)	r= -0.23, p=0.25	r=0.39, p=0.04*	r=-0.002, p=0.99
6-min walk test distance	r=0.05, p=0.82	r=-0.14, p=0.56	r=-0.02, p=0.92
Valentini Disease Activity Index	r=-0.02, p=0.93	r=-0.39, p=0.04*	r=0.16, p=0.41
Body Mass Index	r=0.27, p=0.16	r=-0.16, p=0.41	r=0.53, p=0.002*
Erythrocyte sedimentation rate (mm/h)	r=0.14, p=0.47	r=-0.48, p=0.01*	r=-0.05, p=0.81
C-reactive protein (mg/l)	r=0.2, p=0.31	r=-0.07, p=0.72	r=0.12, p=0.55
C3 complement component (g/l)	r=0.06, p=0.76	r=0.12, p=0.56	r=0.56, p=0.007*
C4 complement component (g/l)	r=-0.07, p=0.73	r=-0.25, p=0.24	r=-0.05, p=0.81

*p < 0.05; HRCT, high resolution computed tomography of the lungs; ILD, interstitial lung disease; DLCO, diffusing capacity of the lung for carbon monoxide

tory disease, RA[17]. These observations lead us to a hypothesis, that increased resistin concentration is the result of severe inflammation, and the patient group in this study did not show high elevation in the systemic markers of inflammation, like ESR or CRP. However, we cannot exclude a bias caused by a limited number of subjects in this study. Further, we did not find an association between resistin level and radiological and functional parameters of the lung involvement. There is convincing literature evidence of the role of resistin in the pathogenesis of PAH associated with SSc[19]. Also the results of Angelini *et al.* suggest that resistin-like molecule (RELM)-beta is involved in the development of PAH in SSc patients[30]. The reason for the lack of relationship between resistin and pulmonary parameters found in our study can be the fact that PAH, significant lung functional impairment and abnormalities in HRCT coexisted in only one patient. However, we found that serum resistin level correlated with the presence of digital ulcers in SSc patients. Although the results indicate a weak correlation, they are concordant with the data of a recent study, which demonstrated a higher prevalence of digital ulcers in SSc patients with elevated serum resistin levels than in those with normal levels[29]. Further, previous results showed that resistin exert direct effect to promote endothelial cell activation by promoting endothelin-1 release[31]. Recently it was also reported that plasma endothelin-1 level was higher in SSc patients with digital ulcers than in a group without them[32]. Our results can thus support a link between resistin and the development of ulcers in SSc patients. They also indirectly implicate a possible role of resistin in the process of aberrant angiogenesis, one of the main features of SSc.

In this study we observed lower serum leptin levels in SSc patients than in healthy subjects, though not

down to significant levels, which is consistent with the results of a previous study[33]. However, literature data focusing on the serum leptin level are controversial. Kotulska *et al*. reported a significantly lower serum leptin level in SSc patients[34]. On the contrary, the results of the two recent studies showed significantly higher levels of circulating leptin in SSc subjects with respect to healthy controls[29,35]. The reason for the conflicting literature data can be heterogeneity of SSc, a limited number of patients, differences in disease duration and activity, and in the treatment of the disease. A correlation of leptin level with C3 and BMI found in this study confirmed previous results obtained in SSc patients[33, 34]. Similarly to the previous studies, we failed to find any association of circulating leptin with other clinical and laboratory parameters of the SSc[33,35]. A relationship between decreased serum leptin and BMI noted in SSc patients can be the result of frequent gastrointestinal tract involvement in this disease. Vernooy *et al*. observed that leptin expression is enhanced in bronchial epithelial cells and alveolar macrophages of ex-smokers compared with never-smokers and they also confirmed the presence of leptin signaling pathway in lung epithelial cells[36]. The observation indirectly supports the correlation between serum leptin levels and smoking found in our study.

Serum adiponectin levels were comparable between SSc and healthy subjects, which is in accordance with the preceding study[20]. Moreover, the investigation of serum adiponectin levels and their association with clinical and laboratory parameters in SSc revealed significantly lower adiponectin levels in patients with active disease as it was evaluated using Valentini activity score[27]. Although the association was weak and should be treated with caution, this finding harmonizes well with the anti-inflammatory and anti-fibrotic functions of this adipocytokine[37]. However, we did not observe statistically significant differences in serum adiponectin concentrations between dcSSc and lcSSc subgroups. It is in conflict with earlier studies, where adiponectin levels were decreased in dcSSc subjects[20]. This discrepancy can be the result of immunosuppressive treatment in more than half of the investigated group, whereas in the cited study patients treated with corticosteroids or other immunosuppressants prior to their first visits were excluded. Further, we did not observe any relationship between circulating adiponectin and radiological and functional parameters of lung involvement except VC. Because the reduction in serum adiponectin levels is associated with the initiation of the fibrotic response, but not with the maintenance, the data from two previous studies reported by the same team of researchers first demonstrated that serum adiponectin levels inversely correlate with the activity of skin sclerosis but not with the severity of ILD (in dcSSc patients), and in the second study serum adiponectin levels were significantly decreased in dcSSc patients with active ILD compared with healthy controls [38]. We found active disease in only 7 out of 29 subjects, and early SSc was present in less than a half of the whole group, which may explain the lack of statistically significant association of adiponectin level with skin and lung fibrosis in our study. However, we cannot exclude the influence of immunosuppressive therapy or a bias caused by the limited number of patients in our study.

CONCLUSIONS

In summary, our results did not establish whether resistin, adiponectin or leptin in SSc patients are involved in the process of lung fibrosis. Instead, we showed that: 1) the concentration of resistin is related to the presence of digital vasculopathy, 2) serum adiponectin reflects disease activity characterized by Valentini disease activity index, and 3) serum leptin is associated with disease duration. To conclude, measurement of serum adipokines can help us to better understand the disease process and further research in this field in a larger sample of patients is of great importance.

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