

Circulating peroxisome proliferator-activated receptor γ is elevated in systemic sclerosis

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Abstract

Introduction: Systemic sclerosis (SSc) is an autoimmune connective tissue disease with distinguished fibrosis of the skin and internal organs. Vascular damage, immune dysregulation and fibroblasts activation contribute to SSc pathogenesis. Peroxisome proliferator-activated receptor γ (PPAR- γ) can be a link between cell metabolism and fibrosis in SSc due to its anti-fibrotic and immunomodulatory properties.

Aim: To measure the serum level of PPAR- γ in SSc patients and correlate it with the SSc subtype, hs-CRP, disease duration, vascular and internal organ involvement.

Material and methods: Twenty-two SSc patients (15 limited SSc, 7 diffuse SSc) matched with healthy controls were analysed. Clinical and laboratory data were collected including specific antibodies, interstitial lung disease, oesophageal involvement, digital pitting scars, disease duration, Raynaud's phenomenon (RP) and modified Rodnan skin score (mRSS). PPAR- γ levels were analysed by ELISA. Statistical analysis was performed with χ^2 , Student's *t*-test and Mann-Whitney-*U* test. Pearson and Spearman correlation analyses were used to establish variables association. The significance threshold was set at $p < 0.05$.

Results: PPAR- γ concentration was elevated in SSc patients in comparison to controls ($p = 0.007$) with the highest difference for diffuse SSc ($p = 0.004$) with significantly elevated mRSS. No association between PPAR- γ levels and hs-CRP, internal organ and vascular involvement, disease duration, autoantibodies and RP onset was found.

Conclusions: The present study revealed elevated serum PPAR- γ in SSc patients, in particular those with a diffuse form, presenting highest mRSS and lowest BMI. Whether circulating PPAR- γ originates from atrophic adipose tissue, reperfused vessels or ischemic tissues needs assessing. Also the biological meaning or effect of elevated serum PPAR- γ requires further studies.

Key words: systemic sclerosis, fibrosis, peroxisome proliferator-activated receptor γ , organ involvement, pits.

Introduction

Systemic sclerosis (SSc) is a serious chronic disease of unknown etiopathogenesis, with the characteristic triad of vascular damage, immune dysregulation and tissue fibrosis. Distinguished skin fibrosis and involvement of internal organs such as lungs, oesophagus, kidneys and heart determine the clinical outcome in systemic sclerosis [1]. Endothelial cells, fibroblasts and lymphocytes dysfunction seem to play an additional role. Genetic factors and environmental exposures contribute to vascular alterations leading to immune activation and progressive tissue fibrosis mediated by different signalling pathways. Recently, the role of dysregulated cell metabolism in fibrotic tissue has been highlighted [2]. Peroxisome proliferator-activated receptor γ (PPAR- γ) can be an interesting link between cell metabolism and fibrosis due

to its anti-fibrotic properties [2, 3]. PPAR- γ is a member of the nuclear receptor superfamily of ligand-activated transcription factors. Upon mitogenic stimulation PPAR- γ can be transported into the nucleus or cytoplasmic compartment where its nuclear function is repressed and nongenomic signalling pathways are being triggered by it [4, 5]. Although PPAR- γ is predominantly expressed in adipocytes, it has been also detected in various cells involved in SSc pathogenesis such as endothelial, fibroblasts and smooth muscle cells [6]. Polymorphisms of the PPAR γ gene are associated with susceptibility to SSc, implying that PPAR- γ can be an important molecular factor in the development of SSc [7]. Apart from a well-established role in the management of lipid and glucose homeostasis, PPAR- γ is involved in regulation of cell maturation, differentiation and synthesis of various mediators contributing to SSc pathogenesis. PPAR- γ attenuates fibrosis by inhibi-

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tion of TGF-β/SMAD signal transduction pathway. On the other hand, transforming growth factor-β (TGF-β) represses PPAR-γ transcription to promote its own pro-fibrotic activity using two independent promoter suppressor domains, TGF-β inhibitory element (TIE) and SMAD-binding elements (SBEs) [8]. Some studies also suggest that the anti-fibrotic effect of PPAR-γ activation may result from upregulation of the tumour suppressor phosphatase and tensin homologue (PTEN), which indirectly antagonizes TGF-β/SMAD signaling pathway [9]. PPAR-γ negatively interferes with nuclear factor κB (NF-κB) pathway and suppresses the inflammatory response. Activation of PPAR-γ polarizes macrophages toward M2 phenotype population of CD163+ cells with anti-inflammatory properties connected with the synthesis of IL-10, IL-6, TGF-β cytokines which demonstrate profibrotic properties [10].

The multifunctional role of PPAR-γ including cell differentiation, immunity, fibrosis and metabolism, makes it a plausible therapeutic target in disorders with underlying PPAR-γ dysfunction. Constitutive activity of PPAR-γ is maintained by endogenous ligands, with many cytokines and adipokines serving as possible connections between adipose tissue, PPAR-γ and SSc pathogenesis [11]. It has been shown that expression and activity of PPAR-γ are impaired in lesional skin and fibroblasts in systemic sclerosis [7] and treatment of mice with PPAR-γ agonist, thiazolidine has reversed organs fibrosis in preclinical models of systemic sclerosis [3]. However, the level of circulating PPAR-γ in systemic sclerosis remains unknown.

Aim

The aim of the study was to evaluate the circulating PPAR-γ in systemic sclerosis and correlate it with the skin and internal organ involvement.

Material and methods

A cohort of 22 patients diagnosed with systemic sclerosis (21 women and 1 man) and 14 healthy volunteers (13 women and 1 man) were included and recruited for the study to assess PPAR-γ concentration in their sera. Both subsets were age, sex, body mass index (BMI) and high-sensitivity C-reactive protein (hs-CRP) matched as presented in Table 1. All patients with SSc fulfilled the American College of Rheumatology/European League Against Rheumatism 2013 classification criteria [12] and were divided into two groups according to the disease subtype: limited cutaneous SSc (lcSSc, n = 15) and diffuse cutaneous SSc (dcSSc, n = 7). Clinical and laboratory data were collected at the time of blood sampling and included the presence of specific antibodies, interstitial lung disease, oesophageal involvement, digital pitting scars and ulcerations, duration of the disease, duration of Raynaud’s phenomenon, assessment of modified Rodnan skin score (mRSS). Antibodies in our patient cohort group were detected and specified as described in our previous paper [13]. In 18 out of 22 (81.81%) SSc patients, coexistence of two or more antibodies was detected with the following incidence: 10 patients out of 22 (45.45%) were anti-topoisomerase I positive (anti-TOPO I), 12 out of 22 were anti-centromere positive (ACA+), against both CENP A+ and CENP B+ (54.55%), 8 (36.36%) patients had antibodies against RP155 (anti-RNA polymerase III), 3 (13.64%) patients were positive for PM-Scl75, and 4 (18.18%) presented Ro-52 antibodies. Anti-Ku antibodies were detected in 2 (9.09%) patients and anti-RP11 in 3 (13.64%) individuals with SSc. Antibodies detected in the patients group are presented in Table 2.

All patients with systemic sclerosis were hospitalized in our department in order to receive rheological

Table 1. Demographic data for PPAR-γ group, including healthy controls and patients with systemic sclerosis (SSc)

Parameter	Controls (n = 14)	SSc (n = 22)	P-value
Age, mean ± SD [years]	53.0 ±11.5	52.68 ±10.29	0.93
Females, n (%)	13 (92.86)	21 (95.45)	0.68
BMI, mean ± SD [kg/m ²]	24.65 ±3.26	23.24 ±3.47	0.23
Disease duration, mean ± SD [years]	N/A	6.64 ±4.6	N/A
Time from the onset of Raynaud’s phenomenon [years]	N/A	10.64 ±6.28	N/A
mRSS	N/A	9.41 ±7.08	N/A
Correlation between PPAR-γ and disease duration	N/A	-0.103932	> 0.05
Correlation between PPAR-γ and mRSS	N/A	0.178153	> 0.05
Correlation between PPAR-γ and time from the onset of Raynaud’s phenomenon	N/A	0.189245	> 0.05
Serum PPAR-γ level, mean ± SD [ng/ml]	13.92 ±1.93	15.98 ±2.2	0.007
hs-CRP level, median (range) [mg/l]	3.47 (0.25–36.91)	1.5 (0.64–2.34)	0.3

BMI – body mass index, mRSS – modified Rodnan skin score, hs-CRP – high-sensitivity C-reactive protein.

Table 2. Antinuclear antibodies detected with immunoblotting in the study group of 22 patients with systemic sclerosis

Antibody type	No. of patients out of 22	Incidence (%)
Anti-TOPO I (Scl-70)	10	45.45
Anti-centromere A	12	54.55
Anti-centromere B	12	54.55
Anti-RP155	8	36.36
Anti-Ro-52	4	18.18
PM-Scl75	3	13.64
Anti-RP11	3	13.64
Anti-Ku	2	9.09

intravenous treatment including sulodexide or alprostadil (PGE1) or to perform routine laboratory and imaging diagnostic tests. In all patients the acute coronary syndrome was excluded based on electrocardiographs (ECG) or echocardiography before the rheological intravenous treatment introduction.

Subjects who had a history of at least one of the following criteria were excluded from the study: acute or chronic renal failure, diagnosis of cancer, stroke, myocardial infarction, infection, diabetes, pregnancy, paroxysmal atrial fibrillation, allergic disorders (e.g. asthma, contact dermatitis, atopic dermatitis), autoimmune and rheumatic disorders other than systemic sclerosis (e.g. systemic lupus erythematosus, pemphigus, pemphigoid, ulcerative colitis, Crohn's disease).

Clinical parameters

Clinical examination of each patient was carefully performed by an independent dermatologist from our clinic. Severity of skin involvement was evaluated using modified Rodnan Skin Score (mRSS). The BMI was calculated as weight/height² (kg/m²). The duration of SSc was estimated by the first noticeable skin thickening of the fingers described as puffy fingers or sclerodactyly. The SSc patients group was divided into short or long disease duration, which was arbitrarily set up for 3 years. Clinical characteristics of patients is presented in Tables 1 and 3.

Interstitial lung disease (ILD) was defined by the presence of lung fibrosis detected on high resolution computed tomography (HRCT) with spirometry and diffusing lung capacity for carbon monoxide (DLCO) results confirming restriction changes. The presence of oesophageal involvement was considered on the basis of reported symptoms regarding difficulties with swallowing, confirmed by a typical outline of this disorder in an X-ray barium swallow study or oesophageal scintigraphy (e.g. dilution or dysmotility of oesophagus).

Radiological images were assessed by experienced radiologists from the radiology department. In order to assess the renal function, estimated glomerular filtration rate (eGFR), blood urea nitrogen and a history of kidney diseases or transplantation were carefully monitored. Vascular involvement was estimated based on clinical signs such as presence of fingertip ulcers or pitting scars. The number of SSc patients with or without organ and vascular hand involvement is shown in Table 4.

Table 3. Demographic data, clinical characteristics and inflammatory parameters referring to the disease subtype and skin involvement in limited cutaneous SSc and diffuse cutaneous SSc, respectively (lcSSc and dcSSc)

Parameter	lcSSc (n = 15)	dcSSc (n = 7)	P-value
Age, mean \pm SD [years]	54.93 \pm 9.32	47.86 \pm 11.32	0.14
Females, n (%)	14 (93.33)	7 (100)	0.69
BMI, mean \pm SD [kg/m ²]	24.24 \pm 2.95	21.1 \pm 3.73	0.04
Disease duration, median (range) [years]	5 (1–15)	7.5 (4–14)	0.06
Duration of Raynaud's phenomenon, median (range) [years]	9.5 (1–30)	11 (5–16)	0.83
mRSS, mean \pm SD	5.47 \pm 1.68	17.86 \pm 6.84	0.001
Serum PPAR- γ level, mean \pm SD [ng/ml]	15.55 \pm 2.18	16.9 \pm 2.1	0.19
hs-CRP level, mean \pm SD [mg/l]	1.4 \pm 0.37	1.59 \pm 0.43	0.29

BMI – body mass index, mRSS – modified Rodnan skin score, hs-CRP – high-sensitivity C-reactive protein.

Table 4. Comparison of PPAR- γ concentration (ng/ml) between patients with and without internal organ and vascular involvement estimated by fingertip ulcerations or pitting scars presence

Clinical symptoms	PPAR- γ concentration, mean \pm SD, comparison		P-value
	With the symptom	Without the symptom	
Interstitial lung disease	n = 11 16.07 \pm 2.4	n = 11 15.88 \pm 2.1	0.84
Oesophagus involvement	n = 12 15.65 \pm 2.16	n = 10 16.37 \pm 2.3	0.46
Presence of fingertip ulcerations or pitting scars	n = 7 16.9 \pm 2.1	n = 15 15.55 \pm 2.18	0.19

Serological methods

Blood samples were collected during routine blood donation in the morning after the overnight fast. Samples were centrifuged within 30 min at room temperature and obtained sera were stored in aliquots at -70°C until processing. Circulating serum levels of PPAR- γ (EIAab, Wuhan, Cat. No. E0886h) were analysed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions. Standards and samples were measured in duplicates and the mean values were calculated to express serum concentrations of PPAR- γ protein in ng/ml.

The study was conducted in compliance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Warsaw (No. KB/66/2018). Informed consent was obtained from all participants included in the study.

Statistical analysis

All statistical analyses were performed using Statistica 13.3 (StatSoft/TIBCO, Cracow, Poland). Each parameter was assessed for normality with Shapiro-Wilk test. Subgroup heterogeneity and dichotomous data were tested with χ^2 test. Student's *t*-test was used to analyse data with normal distribution and Mann-Whitney-*U* test was used for the comparison of non-parametric continuous variables. Pearson and Spearman correlation analyses were used to measure the association between variables. *P*-values < 0.05 were rated as statistically significant.

Results

Demographic characteristics of patients and controls are presented in Table 1. There was no statistically

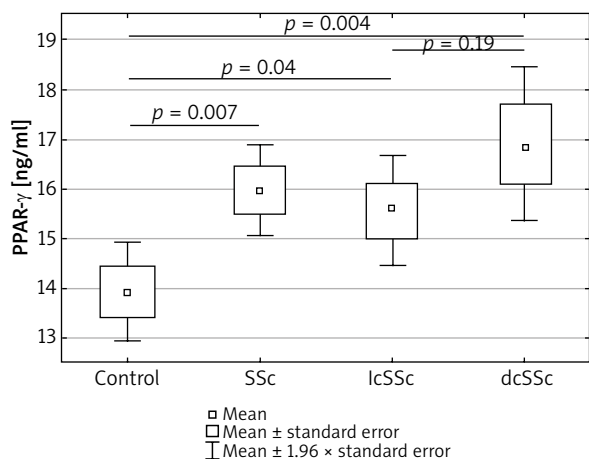


Figure 1. Elevated PPAR- γ concentration in the SSc group vs. healthy control group at $p = 0.007$. The patients group was divided into limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). The most significant difference in PPAR- γ concentration was noticed between healthy and dcSSc at $p = 0.004$, whilst for lcSSc it reached $p = 0.04$

significant difference between the two groups in terms of age, sex, and BMI ($p > 0.05$). Patients with SSc had a significantly higher serum PPAR- γ concentration in comparison to healthy controls at $p = 0.007$ (Figure 1). No associations between PPAR- γ levels and disease duration, duration of Raynaud's phenomenon, and hs-CRP level were found. When the patients group was divided into limited SSc and diffuse SSc types, the lowest BMI was established in diffuse SSc at $p = 0.04$ and is connected to adipose tissue atrophy described in SSc [14], whilst mRSS was significantly elevated in dcSSc at $p = 0.001$ in comparison to lcSSc (Table 3). No other differences were found between lcSSc and dcSSc groups. PPAR- γ mean concentration obtained in dcSSc with the highest skin involvement showed the highest statistical difference in comparison to healthy controls at $p = 0.004$. The difference in PPAR- γ level between the lcSSc group and control group was lower and reached $p = 0.04$ (Figure 1).

No associations were found for PPAR- γ concentration and the presence of ILD, oesophageal involvement as well as pitting scars or ulcerations on the fingertips as presented in Table 4. Serum PPAR- γ concentration correlated negatively, however not significantly, with disease duration in patients without organ involvement. In patients diagnosed with SSc organs complications, rank correlation coefficients revealed no relationship between the PPAR- γ level and disease duration, especially when associated with oesophageal involvement and pitting scars ($R = 0.01$ and $R = -0.01$, respectively, at $p > 0.05$).

Discussion

Peroxisome proliferator-activated receptor γ is a ligand-dependent transcription factor which regulates the expression of genes involved in glucose homeostasis, lipid metabolism, cell growth, inflammation, innate immunity and connective tissue synthesis and degradation [2, 15]. PPAR- γ ligands and inflammation can regulate nucleo-cytoplasmic shuttling and transcriptional activity of PPAR- γ as has been shown in rheumatoid arthritis synovial tissue [16]. The role of PPAR- γ is not clearly established in SSc pathogenesis though it shows anti-fibrotic properties and has been demonstrated to be lower in collagen-rich dermal matrix of scleroderma skin [17]. Our study is the first to assess PPAR- γ concentration in the circulation of patients with systemic sclerosis and finding it significantly elevated. The main concern is the interpretation of this result since aberrant PPAR- γ function has been implicated in pathological fibrosis in the skin and lungs [15] and activation of PPAR- γ exerts an anti-inflammatory and anti-fibrotic effects. Our study revealed the highest concentration of serum PPAR- γ in SSc patients with diffuse SSc, presenting highest mRSS and lowest BMI. The possible interpretation of this result is discussed below.

Majority of studies evaluating serum PPAR- γ concentrations are devoted to cardiology. One of the first studies has revealed a negative correlation between serum PPAR- γ and pro-inflammatory biomarkers such as hs-CRP and IL-6 in atrial fibrillation [18]. Pro-inflammatory cytokines such as IL-6 and hs-CRP have been also reported to be elevated in systemic sclerosis by Ohtsuka [19] and in acute coronary syndrome by Kaminska *et al.* [20]. However, in patients with acute coronary syndrome (ACS), serum PPAR- γ was significantly higher in comparison to the matched controls indicating that it can be used as a diagnostic biomarker for myocardial infarction [21]. In our study, the SSc patients group also showed elevated PPAR- γ which did not correlate with inflammatory biomarkers such as hs-CRP, but with the presence of skin fibrosis.

An elevated circulating level of PPAR- γ in systemic sclerosis may result from adipose tissue loss [14] observed especially in diffuse SSc with increased fibrosis and low BMI as our data have shown. Under physiological conditions, adipose tissue is the most abundant in PPAR- γ , where it is a critical regulator of adipocyte metabolism and differentiation [16]. In case of apparent adipocytes loss in scleroderma [14], PPAR- γ can be released from adipose tissue replaced by fibrosis and reach the highest level in circulation in the diffuse SSc type in which low BMI correlates with intense skin hardening. This can explain the results of our study in which PPAR- γ correlated with skin but not organ involvement or inflammation in SSc.

It has been suggested that serum PPAR- γ concentration serves as a marker of tissue permeability or cell damage rather than as an indicator of the inflammation. PPAR- γ has capability to shuttle between the nucleus and cytoplasm [5] implying that cells do not have to be destroyed in order to release PPAR- γ from the cytoplasmic compartment. Increased and rapid PPAR- γ expression was detected in kidney endothelial cells in response to ischemic injury [22]. Endothelial cell injury due to ischemia is a constant vascular finding in systemic sclerosis [1, 23]. We therefore correlated PPAR- γ with vascular involvement based on Raynaud's phenomenon duration and fingertip ulcerations or pitting scars and could not find any significant association. Therefore the main source of elevated PPAR- γ in SSc patients cannot be endothelial cell injury as has been shown in renal endothelial cells in ischemia-reperfusion injury.

We also revealed that the serum level of PPAR- γ did not correlate with disease duration in SSc ($p > 0.05$). Our results may be extrapolated to another study conducted in systemic sclerosis examining anti-endothelial cell antibodies (AECA). These antibodies did not correlate with disease duration or activity, but with vascular complications in SSc such as digital ulcers, calcinosis and acro-osteolysis [23]. Authors concluded that probably AECA presence reflected constant endothelial damage during the course of SSc and might indicate vascular complication development.

We can only assume that elevated circulating PPAR- γ may reflect adipocytes, endothelium and fibroblast disturbed functions leading to reduced PPAR- γ tissue expres-

sion in scleroderma skin [17] when cell permeability contributes to PPAR- γ release and increased level in circulation. Taking into consideration obtained results together with previously cited references, it remains obscure whether reduced PPAR- γ tissue expression contributes to fibrosis and is mediated e.g. by hypoxia [15] or activated TGF- β signalling [8] or fibrosis is a result of decreased PPAR- γ concentration leading to loss of TGF- β inhibition, fibroblasts to myofibroblasts transition [9] and inflammation. Regardless of the unsolved sequence of events mediated by PPAR- γ , pan PPAR agonist IVA337 was effective in prevention of both lung and skin fibrosis in the mouse animal model of bleomycin-induced pulmonary or dermal fibrosis [17, 24].

An interesting finding is that elevated PPAR- γ may correlate with M2 macrophages, which have been reported elevated in SSc circulation as well [1]. It is known that PPAR- γ activation primes naive human monocytes to differentiate into M2 [10]. M2 macrophages possess anti-inflammatory properties and promote angiogenesis. On the other hand, they produce profibrotic TGF- β , IL-6, IL-10 and are involved in tissue remodelling and SSc pathogenesis [3]. The relationship between elevated circulating PPAR- γ and M2 macrophage shift in systemic sclerosis requires further studies.

Conclusions

The results of our study should be interpreted in light of their limitations. Our sample size is small, but sufficient to show significant differences in PPAR- γ between healthy controls and patients with systemic sclerosis. PPAR- γ does not correlate with inflammatory biomarker such as hs-CRP, but with skin fibrosis as has been shown for diffuse SSc. It needs further studies why anti-fibrotic and immunoregulatory transcription factor such as PPAR- γ is elevated in SSc sera. Significantly elevated PPAR- γ in SSc circulation may indicate its possible role in SSc pathogenesis, but further studies including a greater number of patients are required to fully establish the clinical significance of PPAR- γ and its diagnostic significance in systemic sclerosis.

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Conflict of interest

The authors declare no conflict of interest.

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