

Angiogenin and SDF-1 α serum concentration in patients with systemic sclerosis in relation to clinical status

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Abstract

Introduction: Systemic sclerosis (SSc) is a connective tissue disorder characterized by tissue hypoxia due to vascular changes and excessive fibrosis of the skin and internal organs. Damage to blood vessels and endothelium, as well as imbalance of vascular homeostasis, impairment of angiogenesis and vasculogenesis are observed in the course of the disease. The aim of the study was to investigate the pro-angiogenic factors angiogenin and SDF-1 α in patients with SSc.

Material and methods: Serum samples were collected from 50 patients with dSSc (diffuse SSc) and lSSc (limited SSc) and from 38 patients used as a healthy control group. We explored: 1) how the serum concentrations of SDF-1 α and angiogenin differ in the investigated groups; 2) the correlation among chemokines in SSc and the duration of the disease, Raynaud's phenomenon, sclerosis of the skin and TSS (total skin score).

Results: Patients with SSc showed statistically significantly higher serum angiogenin concentration and there was no correlation between duration of the disease and Raynaud's phenomenon, skin sclerosis or TSS. There was also no difference or no correlation between serum level of SDF-1 α and the investigated groups.

Conclusions: The increase in angiogenin concentration in the serum in patients with SSc may confirm endothelial damage caused by hypoxia and reduced vascular perfusion due to the course of SSc without contributing to compensatory revascularization.

Key words: systemic sclerosis, angiogenesis, vasculogenesis, angiogenin, SDF-1 α .

Introduction

Systemic sclerosis (SSc) is an autoimmune disease in which the following characteristics are observed: vascular changes, sclerosis of the skin and of internal organs. The spectrum of vascular abnormalities extends from sclerosis of the skin to fibrosis of all organs.

It was proven that tissue hypoxia aggravates with the course of the disease due to reduced blood flow and that it induces formation of new blood vessels from the already existing ones (angiogenesis) or from endothelial progenitor cells (EPC) from the bone marrow (vasculogenesis) that circulate in the blood. Survival of tissue destroyed by the vascular autoimmune process is strictly dependent on angiogenesis. There are many factors, such as: modelling the angiogenesis process and formation of new capillaries stimulated by hypoxia, the inflammatory process and pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) [1].

The literature on the subject confirms that regeneration of impaired vessels in the process of autoaggression depends on angiogenesis and also on vasculogenesis [2].

In the course of systemic sclerosis, a decreased number of EPC was described, related to the clinical stage of the disease and found to be directly proportional to the course of the disease [2]. EPC are released by the bone marrow and may play a vital role in formation of new vessels. Stromal cell-derived factor-alpha 1 (SDF-1 α) and VEGF mediate the endothelial progenitor cells' mobilization and differentiation and have a strong influence on regulation of chemotaxis of the CXCR4 (chemokine [C-X-C] receptor 4)-expressing cell population [3]. Interestingly, it was observed that SDF-1 α acts locally within the hypoxic environment and stimulates direct differentiation of the recruited bone marrow cells into endothelial cells [4, 5]. Moreover, it was proved that SDF-1 and CXCR4 were up-regulated in the skin of patients with early (oedematous) SSc, both in the diffuse and limited cutaneous forms, and they progressively decreased with the duration of the disease [6].

Although the pathogenesis of SSc is not completely understood, it is known that both vasculogenesis and angiogenesis are impaired. Angiogenin is one of the most potent pro-angiogenic factors and regulates vessel growth and plays a vital role in the process of angiogenesis and cell proliferation. The protein, exerting its ribonucleic activity, stimulates basement membrane degradation, endothelial cell penetration, migration and formation of tubular vascular structures. However, the angiogenin receptor is not known yet [7-9].

The present study was designed to widen and deepen the problem of angiogenesis disturbing factors in SSc, which might contribute to a better understanding of vascular abnormalities. We hypothesized that the cause of vascular abnormalities in patients with SSc might be correlated with the serum concentration of angiogenin and SDF-1 α , which may have a prognostic implication for the duration and severity of the disease.

Material and methods

Forty-five females and 5 males with SSc (41 ISSc, 9 dSSc) were recruited from the Department of Dermatology and Venereology, Medical University of Lodz. The onset of the disease ranged from 1 to 30 years ago (mean 9 years) and was defined as the time at which skin involvement occurred. The mean age was 53 years (range from 20 to 77 years). The medical history and clinical examination were taken and, if necessary, additional tests were performed (Table I). All patients met the American College of Rheumatology criteria for the classification of SSc [10]. The severity of the disease was assessed according to international guidelines [1]. We measured the TSS (total skin score) according to Kahaleh's score [12]. At the time of serum collection for testing, all patients with SSc were taking vasodilator drugs and corticosteroids (9 patients, 5 with dSSc), methotrexate (2 patients, 1 with dSSc) and cyclophosphamide (2 patients, 1 with dSSc).

Systemic sclerosis patients were compared with the control subjects of the same sex and similar age with no systemic diseases or medication. Blood samples from the patients and the control subjects were collected from 8:00 to 9:00 am after overnight fasting. All samples were collected into trisodium-citrate tubes and sera of all subjects were extracted from the blood samples after centrifugation at 2800 g for 10 min and stored at -20°C until use.

In our study we used the SDF-1 α Immunoassay (R&D Systems, Abingdon, Oxon, UK) and Human Angiogenin Immunoassay (R&D Systems, Abingdon, Oxon, UK) based on quantitative colorimetric sandwich ELISA tests, according to the manufacturer's instructions.

We investigated the levels of the angiogenin and SDF-1 α concentration in the serum and their correlation with the duration of Raynaud's phenomenon, skin sclerosis and TSS.

Statistical significance was analysed by non-parametric Mann-Whitney test, Student's *t*-test and Spearman's rank correlation test. *P* values less than 0.05 were considered significant.

The study was approved by the Ethical Committee of the Medical University of Lodz.

Table I. Clinical characteristic of the patients

		Age			Sex		Reynoud ph				Sclerosis			TSS			
		Min.	Max.	Mean	F	M	ISSc	dSSc	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
SSc	ANG	38	77	54	45	5	41	9	1	30	9	1	20	7	4	40	15
	SDF-1 α	20	70	52	24	1	18	7	1	30	9	1	15	6	4	32	15
Control	ANG	34	68	50	21	4	0	0	0	0	0	0	0	0	0	0	0
	SDF-1 α	21	68	49	16	1	0	0	0	0	0	0	0	0	0	0	0

SSc – systemic sclerosis group, Control – control group, Reynoud ph – Reynoud phenomenon duration, Sclerosis – sclerosis duration, TSS – Total Skin Score, F – female, M – male, ISSc – limited SSc, dSSc – diffuse SSc

Results

Angiogenin

In the investigated groups, higher concentrations of angiogenin in serum were found in SSc patients compared with the control subjects (323.62 ng/ml vs. 255.31 ng/ml; SD 90.39 vs. 39.48). The differences were statistically significant ($p = 0.000082$) (Table II).

There was no statistically significant difference between the patients with ISSc or dSSc taking or not taking immunosuppressive drugs (Tables III and IV).

The differences between serum concentration were not significant ($p = 0.974667$) among the investigated groups – ISSc and dSSc. There was no

correlation with the duration of Raynaud’s phenomenon, skin sclerosis or TSS (Table V).

SDF-1 α

There was no statistical difference in SDF-1 α serum concentration among the investigated groups (Table I).

There was no statistically significant difference between the patients with ISSc or dSSc and whether or not they took immunosuppressive drugs (Tables IV and V).

Although no statistically significant difference or correlation between serum concentration of SDF-1 α in the investigated parameters was found,

Table II. Serum concentration of angiogenin and SDF-1 α among SSc and control groups

	Serum concentration [ng/ml]											
	Angiogenin					SDF-1 α						
	<i>n</i>	Mean	Median	Min.	Max.	SD	<i>n</i>	Mean	Median	Min.	Max.	SD
ISSc	35	322.48	329	28	551	96.05	17	2.594	2.601	2.015	3.675	0.411
dSSc	10	327.60	310	255	495	71.22	7	2.653	2.597	2.223	3.473	0.418
Control	25	255.31	249	177	336	39.48	16	2.746	2.662	2.084	3.954	0.442

Angiogenin: ISSc-dSSc: $p = 0.978219$; ISSc-Control: $p = 0.000684$; dSSc-Control: $p = 0.001230$
 SDF-1 α : ISSc-dSSc: $p = 0.974667$; ISSc-Control: $p = 0.249042$; dSSc-Control: $p = 0.639997$
n – number of patients, SSc – systemic sclerosis group, Control – control group, SD – standard deviation

Table III. Comparison of serum concentration of angiogenin ($p = 0.386449$) and SDF-1 α ($p = 0.342789$) between ISSc patients with/without immunosuppressive therapy

Serum concentration [ng/ml] of angiogenin and SDF-1 α between ISSc taking immunosuppressive drugs						
	<i>n</i>	Mean	Median	Min.	Max.	SD
Angiogenin	7	270.52	276.44	28.80	390.60	119.17
SDF-1 α	5	2.67	2.72	2.26	2.90	0.26
Serum concentration [ng/ml] of angiogenin and SDF-1 α between ISSc and control groups not taking immunosuppressive drugs						
Angiogenin	28	33.54	340.00	216.60	551.00	87.10
SDF-1 α	12	2.56	2.44	2.01	3.67	0.44

Table IV. Comparison of serum concentration of angiogenin ($p = 0.522436$) and SDF-1 α ($p = 0.288852$) between dSSc patients with/without immunosuppressive therapy

Serum concentration [ng/ml] of angiogenin and SDF-1 α between dSSc taking immunosuppressive drugs						
	<i>n</i>	Mean	Median	Min.	Max.	SD
Angiogenin	6	325.10	30.60	255.80	495.00	88.27
SDF-1 α	4	2.81	2.62	2.51	3.47	0.44
Serum concentration [ng/ml] of angiogenin and SDF-1 α between dSSc and control groups not taking immunosuppressive drugs						
Angiogenin	4	331.35	321.40	287.60	395.00	46.90
SDF-1 α	3	2.44	2.27	2.22	2.83	0.33

we observed a tendency for a negative correlation between the serum level of SDF-1 α and the duration of Raynaud's phenomenon ($\rho = -0.270$) and skin sclerosis ($\rho = -0.388$) (Table II). Similarly, we observed a tendency for a negative Spearman's correlation coefficient between SDF-1 α serum concentration and duration of the disease. However, these results were not statistically significant ($p > 0.05$).

Discussion

It seems that in the pathogenesis of systemic sclerosis neovasculogenesis, impairment is a vital element in the development of the disease. According to the literature on the subject, both an increase and a decrease in angio- and vasculogenesis may be a manifestation of the disease [13, 14].

This study confirms the hypothesis of deep impairment of angiogenesis in SSc. It is the first study demonstrating elevated angiogenin concentration in SSc patient serum compared with healthy subjects. Increased release of angiogenin may be indicative of compensatory ineffective angiogenesis in these patients which was not observed in revascularization of the healthy population.

It may also suggest that the process, which is dependent on this molecule, might not be impaired due to the course of SSc, but we hypothesized that the elevated level of angiogenin may occur due to the impairment of endothelial cell differentiation described by Cipriani [15], which showed decreased capacity of specific endothelial activities occurring in angiogenesis. A large decrease in the level of angiogenin reduces the capacity to induce tumour growth and angiogenesis, even in the presence of elevated bFGF (basic fibroblast growth factor) and VEGF [16].

Interestingly, the literature describes elevated angiogenin serum level in chronic heart failure [17], which is related to worsening of heart failure severity. Tello-Montoliu [18] described increased plasma angiogenin level in acute coronary syndromes with predictive adverse events during the follow-up and correlated this protease with the destabilization of atherosclerotic plaque.

In addition to defective angiogenesis, vasculogenesis is also impaired in SSc. Reduced numbers and functional defects of endothelial progenitor cells are observed despite increased endothelial cell activation markers [19, 20].

Considering the insufficient vasculogenesis in SSc patients, up-regulation in SDF-1 α level could be expected, which could have a positive effect on the described lower number of EPC in patients with systemic sclerosis [2, 15]. SDF-1 α takes part in the promotion of recruitment, growth and survival of cells from the bone marrow stromal stem cells [21]. Their participation in tissue regeneration in ischaemic cardiomyopathy is well described.

Table V. Correlation among angiogenin and SDF-1 α serum concentration and the duration of Raynaud phenomenon, skin sclerosis and TSS

		Raynaud ph	Skin sclerosis	TSS
Angiogenin	ρ	0.127	0.096	0.078
	p	> 0.05	> 0.05	> 0.05
SDF-1 α	ρ	-0.270	-0.388	0.058
	p	> 0.05	> 0.05	> 0.05

Raynaud ph – Raynaud phenomenon duration, skin sclerosis – skin sclerosis duration, TSS – Total Skin Score

ρ – Spearman's rank correlation coefficient, $p > 0.05$ – no statistical significant

Surprisingly, we did not observe any statistical significance comparing serum concentration of SDF-1 α in the SSc and healthy control groups. This may suggest that either the homeostasis between SDF-1 α and CXCR4 is maintained or the hypothetical decreased level of CXCR4 [22, 23] does not influence the increased level of SDF-1 α in the serum in our patients. In our studies we observed a tendency for a negative Spearman's correlation coefficient, which might indicate that investigation of a larger group of patients could be positive and it also means that the level of SDF-1 α would decrease with the course of the disease. Interestingly, cell recruitment from bone marrow might even be within normal limits. Furthermore, Cipriani reported a significantly lower expression of CXCR4 in SSc mesenchymal stem cells, thus lowering their differentiation into endothelial cells. Under-expression of CXCR4 may correlate with the duration of SSc by contrast with overexpression, which supports tumour growth and would stimulate excessive angiogenesis [24]. Moreover, Cipriani observed SDF-1 α and CXCR4 up-regulation in the skin of patients with early SSc, dSSc and lSSc, and also progressive reduction of these molecules with the course of the disease, which our study also suggests. The difference between our observations, where the level of SDF-1 α in SSc patient serum is the same as in the control subjects, and the observations described in the literature on up-regulation of SDF-1 α in the skin of SSc patients, may indicate only local impairment of the SDF-1 α /CXCR4 axis [25].

To summarize, a high level of pro-angiogenic factors may indicate disturbances in revascularization homeostasis. Patients with SSc show increased serum concentration of many pro-angiogenic factors including angiogenin, as was shown for the first time in our study, to hypothetically reconstruct blood vessels and increase tissue blood perfusion stimulated by hypoxia.

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