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Association of endothelin-1 and cell surface adhesion molecules levels in patients with systemic sclerosis

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Abstract

Objectives: Endothelin-1 (ET-1) has been implicated in the pathogenesis of inflammatory and fibrotic diseases, including systemic sclerosis. In addition to modulating vascular tone and extracellular matrix turnover, ET-1 up-regulates cell surface adhesion molecules–intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).The aim of the study was to evaluate the diagnostic value of the detection of ET-1, VCAM-1 and ICAM-1 in the diagnosis of systemic sclerosis.

Methods: A total of 30 patients with systemic sclerosis from Vilnius University Hospital Santaros Clinics were included in the study. Serum levels of ICAM-1, VCAM-1 and ET-1 were assessed by enzyme immunoassay.

Results: ET-1 concentration was associated with VCAM-1 concentration ($r=0.687$; $p<0.001$). No associations between ET-1 and ICAM-1 concentrations were detected. Depending on the duration of the disease no significant differences in the concentrations of ET-1, ICAM-1 and VCAM-1 were detected.

Conclusions: The results of this study indicated that ET-1 and VCAM-1 may be assessed together as markers of inflammation and the identification of patients at high risk for disease progression.

Keywords: endothelin-1 (ET-1); intercellular adhesion molecule-1 (ICAM-1); systemic sclerosis; vascular cell adhesion molecule-1 (VCAM-1).

Introduction

Systemic sclerosis (SSc) is a chronic, autoimmune, connective tissue disease, which affects the skin and internal organs. Despite the progress made over the last decade in SSc diagnosis and treatment, disability and mortality from SSc remain the highest among all connective tissue diseases.

SSc is a rare disease, it affects less than one per cent population. Until now, there is no clear etiology of SSc. The disease can be provoked by both genetic predisposition and environmental factors (ultraviolet radiation, chemicals, viral infections), as well as the interaction of these components. The incidence of SSc disease is 3–5 times higher among women. SSc is a disease of young people, usually diagnosed between 30 and 50 years old.

Diagnosis of systemic sclerosis is based on a set of clinical signs, laboratory markers and physical condition. However, no specific laboratory markers have been identified to confirm the diagnosis of SSc and to evaluate the activity and prognosis of the disease. Currently, the main purpose of laboratory tests for diagnosing SSc is to "reject" other possible autoimmune diseases.

Systemic sclerosis causes increased expression of adhesion molecules on the surface of endothelial cells. Soluble forms of these molecules in the blood increases, which can be assessed by laboratory tests using blood serum. Taking into account the biological function and research data, endothelin-1 (ET-1) is considered as one of the major components of SSc pathogenesis. High levels of ET-1 and its receptors are expressed in both endothelial cells and circulating in the blood of SSc patients. Determination of soluble adhesion molecules and ET-1 in serum SSc patients could be one of the laboratory markers for evaluating SSc activity and disease prognosis.

Endothelin system was first purified and sequenced in the late 1980s from cultured endothelial cells [1]. Endothelin secretion is regulated at the gene level. Endothelin

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expression is enhanced by physical factors: hypovolaemia, hypoxia, cold, stress, hormones: angiotensin II, adrenaline, insulin, antidiuretic hormone, cortisol, local factors: growth factors, cytokines (IL-1), activated platelets, endotoxins, thrombin, oxidized low-density lipoproteins [2]. Endothelin gene expression is inhibited by nitric oxide (NO), prostaglandins (PGE1, PGE2), heparin, hepatocyte growth factor (HGF), epidermal growth factor (EGF) and natriuretic peptides [2].

Endothelin family has three endothelin isoforms, ET-1, ET-2 and ET-3. These are peptides made up of 21 amino acid residues. All three have common receptors but have a different affinity. Endothelins stimulate inflammation and proliferation of the vascular inner lining, hypertrophy of the vascular cover, proliferation of fibroblasts, and fibrosis [2].

ET-1 is a vasoconstrictive, anti-inflammatory and proliferative peptide secreted by endothelial cells, important for vascular function regulation. ET-1 is the strongest known vasoconstrictive peptide produced by various cells; most of it is synthesized in the endothelium, epithelial cells, monocytes, macrophages and fibroblasts. The biggest ability to synthesize ET-1 has the endothelium of the small veins, coronary and vascular endothelium.

Considering the biological function and research data, ET-1 is considered one of the major components of SSc pathogenesis. High levels of ET-1 and its receptors are expressed in both endothelial cells and circulating in the blood of SSc patients. Increased ET-1 concentrations were found in the serum of patients with SSc when compared to controls [1, 3]. In patients whose internal organs are damaged by SSc, serum ET-1 levels are significantly higher than in patients who do not yet have systemic lesions [4]. ET-1 plays an important role in the process of vascularization and fibrosis of connective tissue when secreted by the activated and damaged endothelial cells [5].

The vascular cell adhesion molecule-1 (VCAM-1) is a glycoprotein with a molecular weight of 90 kDa. VCAM-1 belongs to the immunoglobulin superfamily. The VCAM-1 gene has six or seven immunoglobulin domains and is expressed in both large and small blood vessels only when endothelial cells are stimulated by cytokines. VCAM-1 mediates adhesion of lymphocytes, monocytes, eosinophils and basophils to vascular endothelium. VCAM-1 is weakly expressed on the endothelial cell surface, but the expression is highly activated by cytokines such as tumour necrosis factor- α (TNF-α), interleukin (IL)-1β, IL-4 or interferon- γ (INF-γ). VCAM-1 secretion is also promoted by hyperglycemia, homocysteine, oxidized low-density lipoproteins and free radicals. Due to its distribution in human tissues and organs, VCAM-1 is involved in many pathophysiological mechanisms and is associated with certain diseases.

The intercellular adhesion molecule-1 (ICAM-1) is a transmembrane glycoprotein with a molecular weight ranging from 60 to 114 kDa. ICAM-1 is not specific to the endothelium, could be found in many cell types–monocytes, lymphocytes, epithelial, smooth muscle, heart muscle cells, keratinocytes, eosinophils, fibroblasts, dendritic cells, hepatocytes, stem cells. The expression of ICAM-1 is stimulated by cytokines such as IL-1, TNF-α, INF-γ. The expression is also promoted by bacterial endotoxins and oxidation of low-density lipoproteins.

VCAM-1 and ICAM-1 adhesion molecules are widely used as endothelial dysfunction markers. The importance of adhesion molecules in the pathogenesis of SSc is illustrated in several scientific studies [6, 7]. In the early stages of SSc, increased serum concentrations of soluble adhesion molecules are detected in patients and especially in those who are actively progressing. Even at the onset of the disease, there is an increased adhesion molecule in the skin of SSc patients [8]. Soluble adhesion molecules are also found in the blood. Increased VCAM-1 concentration in serum may be related to the extent of organ damage in the SSc [6].

This research aimed to evaluate the diagnostic value and influence of ET-1, VCAM-1 and ICAM-1 in the serum of SSc patients.

Materials and methods

A total of 30 SSc patients were involved in this study. Patients were treated in Vilnius University Hospital Santaros Clinics. Systemic sclerosis was diagnosed based on classification criteria of the American Rheumatology College (ACR) and the European League Against Rheumatic Diseases (EULAR). Serum samples were collected from each patient. The majority of patients were women (n=26). The mean age of the subjects was 52.5 ± 8.7 years. Twenty five subjects were diagnosed with a limited form of SSc, others had diffuse SSc. Patients were divided into three groups according to disease duration: less than five years (n=17); 5–10 years old (n=6); more than 10 years (n=17). Eleven subjects were diagnosed with pulmonary arterial hypertension (PAH). Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and was approved by the Vilnius University Bioethics Committee (Decision No.: 158200-15-800-310). Informed consent was obtained from all individuals included in this study.

Enzyme-linked immunosorbent assay (ELISA) was performed to measure VCAM-1, ICAM-1 and ET-1 levels, following the manufacturer's instructions. ELISA kits for Human VCAM-1, ICAM-1 and ET-1 was from IBL International, Germany. Samples were analyzed in duplicate and the optical densities were determined at 450 nm.

Average recommended concentration of ICAM-1 in healthy subjects is <504 ng/mL, VCAM-1 is <772 ng/mL and ET-1 is <0.65 pg/mL.

Statistical analysis

Statistical data analysis was performed using Microsoft Office Excel 2013 and IBM SPSS Statistics 21 software packages. Qualitative data were described as absolute numbers and percentages. The arithmetic mean and the standard deviation were used to describe the parametric data. Non-parametric data were characterized by mean, minimum, and maximum values. Kolmogorov-Smirnov's criteria were used to determine the normal distribution of variables. The correlation coefficient of Spearman was used to evaluate the interfaces between biological markers (ET-1, ICAM-1 and VCAM-1). Differences in data considered statistically significant when the p-value of the error probability was less than 0.05 ($p \leq 0.05$).

Results

The mean ET-1 concentration was 4.86 pg/mL, with a minimum of 0.435 pg/mL, and a maximum of 67.95 pg/mL. Results of ET-1 concentration in 25 patients was above the reference value recommended by the reagent manufacturer (<0.65 pg/mL) (Figure 1). The mean ICAM-1 serum concentration was 372.39 ng/ml (SD=106.19). Twenty two of 25 patients showed lower ICAM-1 levels than the manufacturer's recommended mean concentration (<504 ng/mL) (Figure 1).

Marked red is the reagent manufacturer recommended mean concentration value. VCAM-1 and ET-1 concentrations was above the reference value recommended by the reagent manufacturer. The mean ICAM-1 serum concentration was lower than the manufacturer's recommended mean concentration.

The study showed a statistically significant positive relationship between ET-1 and soluble adhesion molecule VCAM-1 concentration in the serum of the subjects ($r=0.687$; $p<0.001$). There was no statistically significant correlation between ET-1 and ICAM-1 concentration. There were no statistically significant differences in ET-1, ICAM-1, and VCAM-1 concentrations depending on disease duration (Table 1). We did not find any statistically significant differences between patients diagnosed with PAH and without PAH. The mean concentration of VCAM-1 was 1137.75 ng/ml, with a minimum of 457.0 ng/mL and a maximum of 6202.5 ng/mL.

Discussion

The occurrence of endothelial dysfunction and fibrosis during SSc indicates that endothelial cell secreting agents such as ET-1 may be important factors in the pathogenesis of systemic sclerosis and may be used as diagnostic markers [9]. ET-1 is an endogenous vasoconstrictor secreted by vascular endothelial cells. ET-1 promotes endothelial proliferation, fibrosis, and vascular inflammation [9]. Twenty five subjects in our study had elevated serum ET-1 concentration above the reference mean value (<0.65 pg/mL). This increase in ET-1 concentration during SSc can lead to skin thickening, fibrosis and vascular damage [10]. Other studies like the one conducted in 2013 also showed that in patients with SSc, ET-1 levels were higher than in control group [11]. Our study included only patients with SSc, so no differences between healthy subjects and SSc patients could be reported. The results of this study coincide with those of other authors.

Table 1: Differences in ET-1, ICAM-1, and VCAM-1 mean concentrations depending on disease duration.

Disease duration	ET-1, pg/ml	p-Value	VCAM-1, ng/ml	p-Value	ICAM-1, ng/ml	p-Value
<5 years	4,75	0.387	1180.50	0.19	325.00	0.19
5–10 years	5.03		924.25		411.95	
>10 years	14.5		1322.00		344.3	

ET-1, endothelin-1; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.d.

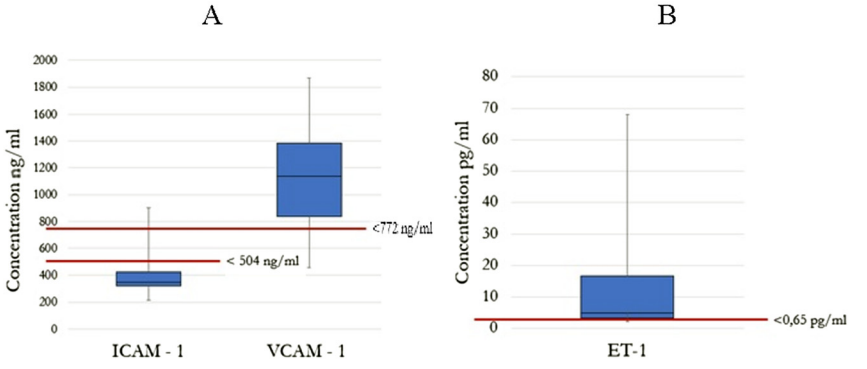

Figure 1: Comparison of soluble adhesion molecules (ICAM-1, VCAM-1) (A) and ET-1 concentrations (B) and in blood serum of patients.

Soluble adhesion molecules (ICAM-1, VCAM-1) are considered as potential SSc biological markers because their concentration in the blood is related to endothelial activity, damage and may reflect endothelial activation status [12]. The importance of adhesion molecules in SSc has been described in several studies, but many of them are based on small groups [11]. In our study, ICAM-1 concentration did not exceed the average concentration recommended by the reagent manufacturer. It is interesting result since in literature ICAM-1 concentration in subjects with SSc were found to be significantly higher than in the control groups [11]. The average concentration of VCAM-1 (1351.82 ng/ml) found in our study was almost twice the recommended mean concentration (<772.2 ng/mL) of the reagent manufacturer. Examining literature we came across one study that found that in SSc patients ICAM-1 and VCAM-1 concentrations were significantly higher than those in healthy controls [13] but in recent paper differences in VCAM-1 levels between healthy and SSc patients were not found [14]. According to literature, ET-1 stimulates VCAM-1 secretion [15, 16]. The non-elevated ICAM-1 in our study could show some underlining individual differences between patients or differences of the treatment protocols. According to literature there is little evidence for steroids to affect concentrations of adhesive molecules [17]. This study shows an importance of individual answer of patient's endothelia to the disease. Such research coincides with the findings of our study and confirms the involvement of both ET-1 and VCAM-1 in the pathogenesis of SSc.

Our study tested the correlation between PAH and the concentrations of adhesion molecules. In literature some studies agree with these results [18], but others would disagree [19]. It would seem that this question is not clear and should be answered and clarified. Our study indicates that PAH does not increase the concentrations of vascular adhesive molecules in SSc patients. This could be important in drawing future prognosis and assessing risks of further complications of disease.

Conclusions

There was a statistically significant relationship between serum ET-1 and VCAM-1 concentration ($r=0.687$; $p<0.001$). The results of this study confirmed the involvement of ET-1 and VCAM-1 in the pathogenesis of systemic sclerosis and these markers can be used to assess the activity of the inflammatory process in the systemic sclerosis. This could allow early identification of patients at high risk of disease complications.

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Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and was approved by the Vilnius University Bioethics Committee. (Decision No.: 158200-15-800-310).

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