Rheumatology: Current Research

Erten, et al., Rheumatology (Sunnyvale) 2015, 5:4
DOI: 10.4172/2161-1149.1000171

Research Article Open Access

The Effect of Oxidative Stress on Pulmonary Involvement in Patients with Systemic Sclerosis

Sukran Erten[1], Tahsin Murat Turgay[1], Erdinc Devrim[2], Serdal Kenan Kose[3], Berrin Imge Erguder[2], Ilker Durak[2], Orhan Kucuksahin[1*], Ali Sahin[1], Alexis K OKOH[1] and Emre Kulahcioglu[1]

[1]*Clinical Immunology and Rheumatology, Ankara University School of Medicine, Ankara, Turkey*

[2]*Biochemistry, Ankara University School of Medicine, Ankara, Turkey*

[3]*Biostatistics, Ankara University School of Medicine, Ankara, Turkey*

[*]**Corresponding author:** Orhan Kucuksahin, Department of Rheumatology, Ankara University Medical Faculty, Ankara, Pınarbaşı mah avcı sokak divan konutları A/5 blok daire 44 Keçiören, 06540, Ankara, Turkey, Tel: 90 312 441 2886; Fax: 90 312 3097779; E-mail: orhankcs@gmail.com

Received date: September 09, 2015; **Accepted date:** October 10, 2015; **Published date:** October 20, 2015

Abstract

Introduction: The aim of this study was to evaluate the relationship between oxidative stress and pulmonary involvement and its severity in patients with systemic sclerosis (SSc).

Materials and Methods: 34 patients (30 female, 4 male) and 27 healthy volunteers (21 female, 6 male) were included into the study. All patients fullfilled the American College of Rheumatology criteria for the diagnosis of SSc.

Oxidant-antioxidant enzymes malondialdehyde (MDA), Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), xanthine oxidase (XO), and adenosine deaminase (ADA) enzyme activities were measured by spectrophotometric methods.

Results: Plasma ADA levels was significantly higher in patients than the control group. On contrary, XO levels of erythroctes were lower. Of 34 patients, 11 had no pulmonary involvement, 14 had mild to moderate and 9 had severe-end stage pulmonary involvement. In patients with pulmonary involvement, plasma XO level was statistically significantly lower but intraerythrocyte XO levels were higher (not statistically significant) than the patients without pulmonary involvement. Severe-end stage group had significantly higher intraerythrocyte XO activity. In limited early disease, intraerythrocyte SOD level was significantly lower than the other groups. Plasma MDA level was significantly higher in limited early and diffuse early disease. Plasma MDA, GSH-PX and intraerythrocyte ADA levels were negatively correlated with disease duration. Intraerythrocyte MDA level was positively correlated with pulmonary arterial pressure.

Conclusions: This study may show that besides the role in the pathogenesis of systemic sclerosis, oxidative stress may also play a role in the severity of the pulmonary involvement of the disease.

Keywords: Systemic sclerosis; Scleroderma; Oxidative stress; Pulmonary involvement

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by fibrosis of the skin, blood vessels, skeletal muscles, and visceral organs and associated with some immunological abnormalities and vascular injury [1]. Collagen overproduction by activated stromal fibroblasts, autoantibody production and acral vasospasm known as Raynaud's phenomenon are the hallmarks of the disease [2]. There are two major subgroups: limited cutaneous SSc and diffuse cutaneous SSc. The kidneys, esophagus, heart, and lungs are the most frequent targets [3].

Under normal circumstances there is a balance between oxidant agents and antioxidant defense mechanisms, and if this balance is disturbed oxidative stress occurs. Reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-) are known as oxidant agents [4]. Malondialdehyde (MDA) as a lipid peroxidation end product is an indicator of oxidative stress [5] Xanthine oxidase (XO) is an oxidant enzyme catalyzing oxidation of hypoxanthine to xanthine and xanthine to uric acid, and can produce O_2^- during these reactions [6]. Superoxide dismutase (SOD) catalyzes the dismutation of O_2^- to hydrogen peroxide (H_2O_2), and catalase (CAT) and glutathione peroxidase (GSH-Px) are the enzymes that catalyze the reduction of H_2O_2 to water. SOD, CAT, and GSH-Px are known as endogenous antioxidant enzymes playing important role in antioxidant defense [7]. Adenosine deaminase is a key enzyme in the degradation of adenine nucleotide [8].

Free radical mediated damage could be an important basis for the SSc pathogenesis [9]. Oxidative stress is supposed to play a role on endothelial injury at earlier stages of the disease [10]. Modifications of the vascular system leads to loss of the control of vascular tone [11]. The oxygen free radical species can be generated during Raynaud's phenomenon that causes hypoxic/ischemic episodes, with consequent lipid peroxidation and tissue damage [12]. Free radicals are harmfull to cells, modifying cellular macromolecules including lipids, proteins,

carbohydrates and nucleic acids [13]. Variation in prooxidant or antioxidant genes may also be associated with SSc [14].

There is evidence of an association between oxidative stress and SSc from several studies [15-27]. Lipid peroxidation products, which are markers of oxidative stress were found at highest levels in patients with interstitial lung disease and in patients with frequent ischemia/reperfusion episodes [24]. Inflammation can play a predominant role in the generation of reactive oxygen species. Tissue damage by respiratory burst of polymorphonuclear leucocytes can occur especially in lungs [26]. The oxygen free radicals are supposed to contribute to the tissue damage occuring in diffuse lung diseases, a heterogenous group of diseases with pulmonary fibrosis of various degrees of severity [28]. Pulmonary involvement (interstitial lung disease and pulmonary hypertension) and cardiac involvement are major causes of death in SSc [29]. Therefore, the aim of this study was to to measure levels of oxidant and antioxidant enzymes in patients with Ssc, to evaluate possible contibution of oxidative stress to the pathogenesis of SSc and pulmonary involvement and its severity.

Materials and Methods

This study was approved by the Ethics Committee, Ankara University School of Medicine and was in accordance with the Helsinki Declaration of 2002.

The study included 34 patients (30 female, 4 male) with systemic sclerosis who were attended to Rheumatology Department for their disease and 27 healthy volunteers (21 female, 6 male) as control group. Patients with SSc fulfilled the American College of Rheumatology criteria for the disease [30] and were classified as having either diffuse or limited disease as described [31] Disease stages were defined as suggested by Medsger and Steen [32]: early limited SSc, disease duration <5 years; late limited SSc, disease duration ≥ 5 years; early diffuse SSc, disease duration<3 years; late diffuse SSc, disease duration ≥ 3 years. Patients with other chronic diseases like diabetes mellitus, coronary artery disease, hypertension and hypercholesterolemia, patients with primary lung diseases or systemic diseases which may affect pulmonary functions were not included. Cigarette smoking was not allowed. Patients were living in central Anatolia region of Turkey and had similar socioeconomic status and dietary habits. Patients were using a wide range of medications like corticosteroids, antihypertensives (nifedipine), colchicine, prostaglandin agonists, acetyl salicylic acid, oral iron preperations, biphosphonates, elementary calcium and vitamin D and cyclophosphamide.

After giving the informed consent, blood samples were taken and chest X-rays, electrocardiography, respiratory function tests and carbon monoxide diffusion tests, high resolution computerized tomography of the lung and Doppler echocardiography were performed. Severity of pulmonary involvement was classified according to Medsger et al. [33].

Fasting blood samples were obtained from the participants in anticoagulated tubes (with EDTA), plasma and erythrocyte hemolysates were obtained. MDA levels, and SOD, CAT, GSH-Px, XO and ADA activities were measured in the erythrocyte hemolysates and MDA levels, and GSH-PX and ADA activities were measured in the plasma in order to establish oxidant/antioxidant status intracellularly end extracellularly, respectively.

Malondialdehyde (MDA) levels were measured by the thiobarbituric acid reactive substances method [34]. Xanthine oxidase activity was determined by measuring uric acid formation from xanthine substrate at 293 nm [35]. Glutathione peroxidase (GSH-Px) activity was measured by following changes in NADPH absorbance at 340 nm [36]. Catalase (CAT) activity was determined by measuring decrease of H_2O_2 absorbance at 240 nm [37]. In the activity calculations (IU-international unit), extinction coefficients of uric acid, H_2O_2 and NADPH were used for XO, CAT and GSH-Px, respectively. Superoxide dismutase activity was measured by the method based on nitro blue tetrazolium (NBT) reduction rate. One unit for SOD activity was expressed as the enzyme protein amount causing 50% inhibition in the NBT reduction rate [38]. Adenosine deaminase (ADA) activities were measured by the method of Giusti [39].

Statistics

The statistical package for social sciences (SPSS) version 11.5 was used for the statistical analysis. For the comparison of measured variables, Student's t test was used for parametric variables and Mann-Whitney U test was used for nonparametric variables. For evaluation of the data for disease severity, one-way variance analysis was used for parametric variables and Kruskall-Wallis variance analysis was used for nonparametric variables and Chi-square and Fisher's exact tests were used for counted variables to determine the significant differences between the groups. Spearman's Rank Correlation Coefficient was used to compare the relationship between two continuous variables. Data were expressed as arithmetic mean ± SD for measured parameters and expressed as frequency and percent for counted parameters. P values <0.05 were considered as significant.

Results

The SSc patients and control group were age and sex matched (51.86 ± 12.19, 55.06 ± 12.19 respectively). Of 34 patients, 30 were female (88.2%) and 4 were male (11.8%). In the control group, 21 were female (77.8%) and 6 were male (22.2%). Mean disease duration was 10.28 ± 8.06 years. Of 34 patients, 17 patients had limited and 17 patients have diffuse form of the disease. Some clinical and laboratory parameters of the patients were shown on Table 1.

Patient features	Mean value ± sd (min-max)
Age(year)	51.86 ± 12.2(25-74)
Disease duration(year)	10.28 ± 8.06 (1-34)
Hemoglobin(g/dl)	12.38 ± 2.0 (5.4-15)
Leucocyte count (4500-11000)	6859.4 ± 20.92.1 (4400-12800)

C-reactive protein (0-3 mg/dl)	9.3 ± 16.8 (0.4-98.5)
Erythrocyte sedimentation rate(0-20 mm/hr)	27.7 ± 26 (2-115)
Sex (Female/Male)	30/4 (%88.2 vs. %11.8)
Disease subsets	
Limited disease	17 (%50)
Diffuse disease	17 (%50)
Autoantibody positivity	
Anticardiolipin antibody(ACA)	9 (%26.47)
Anti-Scl 70	17 (%50)
Antinuclear antibody (when ACA ve Anti-Scl 70 were negative)	10 (%29.4)
SD: Standard Deviation	

Table 1: Clinical and demographic features of 34 patients with systemic sclerosis.

Of 34 patients, 11 patients had no pulmonary involvement, 14 patients had mild to moderate and 9 patients had severe or end-stage pulmonary involvement in accordance with Medsger classification (Table 2).

Pulmonary involvement	
Absent	11 (%32.35)
Mild-moderate	14 (%41.17)
Severe-end stage	9 (%26.47)
Pulmonary hypertension	
Absent	28 (%82.3)
Present	6 (%17.64)
Forced vital capacity(%)	94.59 ± 19.55 (43-130)
Diffusion capacity(DLCO)(%)	64.62 ± 22.17 (22-110)
Pulmonary pressure(%)	20 ± 10.57 (20-65)
High resolution computerised tomography	
Normal	12
Active fibrosing alveolitis	18
Honeycomb appearance	4

Table 2: Pulmonary findings of 34 patients with systemic sclerosis.

Plasma ADA level was significantly higher in patients than the control group ($p<0.05$). On contrary, XO level of erythrocytes were lower in patients than the control groups ($p<0.05$) (Table 3).

Patients with pulmonary involvement had significantly lower plasma XO level. Although it was not statistically significant, XO level in erythrocytes was higher in patients with pulmonary involvement than the patients without pulmonary involvement (Table 4).

Enzyme levels	**SSC patients (n=34)**	**Control group (n=27)**	**p**
a-Oxidant enzymes			
MDA(e) (nmol/ml)	285.06 ± 34.34	289.29 ± 51.51	p>0.05
ADA(e) (mIU/ml)	202.11 ± 100.35	259.72 ± 150.44	p>0.05
XO(e) (mIU/ml)	0.92 ± 0.57	1.2 ± 0.45	p<0.05
MDA(p) (nmo/ml)	0.88 ± 0.53	1.26 ± 0.8	p>0.05
ADA(p) (mIU/ml)	21.39 ± 26.5	15.58 ± 6.3	p<0.05
XO(p) (mIU/ml)	0.14 ± 0.04	0.15 ± 0.03	0.15 ± 0.03
b-Antioxidant enzymes			
CAT(e) (IU/ml)	49876.97 ± 7591.36	48420.44 ± 6594.89	p>0.05
GSHPX(e) (IU/ml)	30.25 ± 2.26	28.98 ± 7.25	p>0.05
SOD(e) (U/ml)	2300.7 ± 520.31	2293.49 ± 429.61	p>0.05
GSHPX(p) (IU/ml)	0.52 ± 0.03	0.52 ± 0.18	p>0.05
MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; GSH-PX: Glutathione Peroxidase; XO: Xanthin Oxidase; ADA: Adenosine Deaminase; E: Erythrocyte; P: Plasma			

Table 3: Comparison of oxidant-antioxidant enzyme activities between SSc patients and the control group.

Enzyme levels	**Pulmonary involvement absent (n=11)**	**Pulmonary involvement present (n=23)**	**p**

a-Oxidant enzymes			
MDA(e) (nmol/ml)	274.88 ± 35.65	287.71 ± 34.29	p>0.05
ADA(e) (mIU/ml)	256.53 ± 69.84	187.27 ± 103.48	p>0.05
XO(e) (mIU/ml)	0.63 ± 0.42	0.99 ± 0.59	p<0.05
MDA(p) (nmo/ml)	0.7 ± 0.53	0.92 ± 0.53	p>0.05
ADA(p) (mIU/ml)	14.1 ± 10.59	23.47 ± 29.39	p>0.05
XO(p) (mIU/ml)	0.16 ± 0.49	0.13 ± 0.03	p<0.05
b-Antioxidant enzymes			
CAT(e) (IU/ml)	52338 ± 5467.99	49234.96 ± 8029.18	p>0.05
GSHPX(e) (IU/ml)	29.64 ± 1.08	30.40 ± 2.48	p=0.06
SOD(e) (U/ml	2297.68 ± 263.28	2301.52 ± 575.82	p>0.05
GSHPX(p) (IU/ml)	0.53 ± 0.02	0.51 ± 0.03	p>0.05
MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; GSH-PX: Glutathione Peroxidase; XO: Xanthin Oxidase; ADA: Adenosine Deaminase; e: erythrocyte; p:plasma			

Table 4: Comparison of oxidant-antioxidant enzyme activities between SSc patients with pulmonary involvement.

In patients with severe or end stage pulmonary disease, XO level in erythrocytes was significantly higher than the other groups ($p<0.05$) (Table 5).

Enzyme levels	No involvement (n=11)	Mild-moderate (n=14)	Severe-end stage (n=9)	p
a-Oxidant enzymes				
MDA(e) (nmol/ml)	274.88 ± 35.65	289.76 ± 37.46	284.52 ± 30.59	p>0.05
ADA(e) (mIU/ml)	256.53 ± 69.84	196.96 ± 120.6	202.11 ± 100.35	p>0.05
XO(e) (mIU/ml)	0.63 ± 0.42	0.79 ± 0.5	1.3 ± 0.62	p<0.05
MDA(p) (nmo/ml)	0.7 ± 0.53	0.88 ± 0.52	0.99 ± 0.58	p>0.05
ADA(p) (mIU/ml)	14.1 ± 10.59	17.12 ± 14.74	31.94 ± 41.5	p>0.05
XO(p) (mIU/ml)	0.16 ± 0.49	0.13 ± 0.04	0.13 ± 0.02	p>0.05
b-Antioxidant enzymes				
CAT(e) (IU/ml)	52338 ± 5467.99	48521.14 ± 9036.93	50345.33 ± 6503.87	p>0.05
GSHPX(e) (IU/ml)	29.64 ± 1.08	30.39 ± 2.58	30.43 ± 2.46	p=0.06
SOD(e) (U/ml	2297.68 ± 263.28	2379.52 ± 308.7	2165.02 ± 885.59	p>0.05
GSHPX(p) (IU/ml)	0.53 ± 0.02	0.52 ± 0.02	0.51 ± 0.04	p>0.05
MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; GSH-PX: Glutathione Peroxidase; XO: Xanthin Oxidase; ADA: Adenosine Deaminase; e: erythrocyte; p: plasma				

Table 5: Comparison of oxidant-antioxidant enzyme activities of SSc patients with severity of pulmonary involvement.

9 patients have limited early disease, 8 had limited late disease, 3 patients had diffuse early and 14 patients had diffuse late disease. In limited early disease, SOD level in erythrocytes was significantly lowers than the other groups. Also, both in limited early and diffuse early disease, MDA level in plasma was significantly higher than the other groups (Table 6).

Enzyme levels	Limited early (n=9)	Limited late (n=8)	Diffuse early (n=3)	Diffuse late (n=14)	p
a-Oxidant enzymes					
MDA(e) (nmol/ml)	293.4 ± 37.3	271.7 ± 12.2	302.4 ± 24.2	286.6 ± 43	p>0.05
ADA(e) (mIU/ml)	254 ± 76.2	208.1 ± 110.3	182.8 ± 115.4	186.9 ± 103	p>0.05
XO(e) (mIU/ml)	0.8 ± 0.7	1 ± 0.4	1 ± 0.2	0.9 ± 0.7	p>0.05
MDA(p) (nmol/ml)	1.4 ± 0.4	0.7 ± 0.6	1.3 ± 0.5	0.7 ± 0.5	p<0.05
ADA(p) (mIU/ml)	18.8 ± 16.6	17.6 ± 9.8	11 ± 2.9	27.4 ± 37.9	p>0.05
XO(p) (mIU/ml)	0.1 ± 0.01	0.15 ± 0.04	0.13 ± 0.01	0.13 ± 0.04	p>0.05
b-Antioxidant enzymes					
CAT(e) (IU/ml)	46390 ± 3223.8	52246.5 ± 7666.7	53558 ± 7193.9	48730.3 ± 8389	p>0.05
GSHPX(e) (IU/ml)	0.9 ± 2.1	29.8 ± 1.4	30 ± 2	30.4 ± 2.8	p>0.05
SOD(e) (U/ml)	1628 ± 1001.8	2335.2 ± 248.8	2443.4 ± 400	2453.5 ± 336.8	p<0.05
GSHPX(p) (IU/ml)	0.5 ± 0.02	0.5 ± 0.03	0.6 ± 0.0	0.5 ± 0.02	p>0.05
MDA: Malondialdehyde; SOD: Superoxide Dismutase; CAT: Catalase; GSH-PX: Glutathione Peroxidase; XO: Xanthine Oxidase; ADA: Adenosine Deaminase; e: erythrocyte; p: plasma					

Table 6: Oxidant-antioxidant enzyme activities in early and late disease.

Plasma MDA and GSH-PX levels and also ADA level in erythrocytes were negatively correlated with disease duration (r=-0.379, p=0.04; r=-0.356, p=0.05; r=-0.357, p=0.05 respectively). MDA level of erythrocytes was positively correlated with pulmonary arterial pressure (r=0.406, p=0.049).

Discussion

In this study, we have shown that oxidative stress may play a role in the pathogenesis of systemic sclerosis and shows its effect mostly at earlier stages of SSc and this effect decreases with time. Oxidative stress may also play a role in pulmonary involvement and its severity.

Murrel et al have proposed that superoxide radical production by way of xanthine oxidase reaction may play a role in pathogenesis of SSc [40]. During ischemia, xanthine dehydrogenase is converted to xanthine oxidase, and the substrates hypoxanthine and xanthine were released with destruction of adenosine triphosphate. When oxygen is supplied with reperfusion, superoxide radicals and hydrogen peroxide appear [15]. Since endothelial cells lack catalase, which is an antioxidant enzyme, they are susceptible to oxidative stress caused by reactive oxygen species. As a result, endothelial cell damage, including intimal proliferation and fibrous thickening of media occurs [19]. Increased xanthine oxidase activity in erythrocytes of SSc patients with pulmonary involvement and in severe or end-stage pulmonary disease demonstrates that oxidative injury is also associated with pulmonary involvement and its severity. Positive correlation of intraerythrocyte MDA levels, 'which is a common biomarker for lipid peroxidation' with pulmonary pressure also supports this.

The relationship between oxidative stress and worsening of pulmonary functions was shown by some other studies [24,26,41-43]. In a study, increased lipid peroxidation in bronchoalveolar lavage fluid of patients with SSc and fibrosing alveolitis was demonstrated [41]. In another study, increased F2 isoprostane levels in lung tissue sections and urine samples of patients with pulmonary hypertension were shown [43]. Solans et al have shown that levels of lipid peroxidation products were higher in SSc patients with interstitial lung disease and concluded that free radical mediated damage may be involved in the structural and functional pulmonary changes present in SSc [9]. Musellim and colleagues have demonstrated that oxidant burden was increased in SSc patients with interstitial lung involvement. However, they could not show any relation between oxidant-antioxidant levels and severity of lung involvement [27].

Endothelial dysfunction is seen mostly at earlier stages of the disease. Inflammatory cell activation is most prominent at earlier stages of the disease when ischemic attacks are more frequent [9,13,21]. In the present study, increases in plasma MDA levels in the limited early and diffuse early disease, and decreases in intraerythrocyte antioxidant enzyme SOD levels in the limited early disease supports that endothelial dysfunction in SSc is an early event. Also, oxidative stress may play an important role in initiation of the vascular disease and much prominent in limited form of the disease. Negative correlation of plasma MDA, GSH-PX levels and ADA levels in erythrocytes with disease duration also demonstrates that oxidative stress in SSc is an early event and may play a role in disease pathogenesis.

Increases in plasma ADA levels are seen due to release of the enzyme from activated T cells. ADA is a ubiquitous enzyme that plays a role in purine metabolism where it deaminates adenosine to inosine. Similar to the present study, Emerit et al also found increased ADA levels in patients with SSc [15].

Changes in SOD activity was shown by some other studies. Morita et al have shown increased SOD activity which was associated with skin sclerosis and Raynaud's phenomenon [44]. Emerit et al. and Musellim et al. have not shown any difference between SOD levels of SSc patients and the control group [15,27]. The conflicting results may

be due to measurement of the enzyme at different stages of the disease. Increased production of superoxide at early stages of SSc may be followed by consumption of the enzyme. Also, decrease in antioxidant defence mechanisms may have some contribution to disease pathogenesis. The results may be affected by the drugs used by the patients like corticosteroids or calcium-channel blockers or the small sample size.

Since lipid peroxidation in SSc is much higher at early stages of the disease and decreases with time, antioxidant treatment might be more effective at early stages of the disease. Denton et al have shown that treatment with probucol is useful in SSc for Raynaud's phenomenon and also reduces low density lipoprotein oxidation susceptibility [45]. Allanore et al have shown that dihydropyridines significantly decreased oxidative stress in patients with SSc. They also demonstrated that oxidative stress in SSc is consistent with the superoxide overproduction by primed monocytes and this was decreased by nifedipine treatment [46, 47].

The most important restriction of this study is the small sample size. Also, the medications used by the patients may have antioxidant properties. Further prospective studies with large number of patients are necessary.

In conclusion, oxidative stress in SSc is an early event and may play a role in disease pathogenesis. It may also have a role in severity of pulmonary involvement. Antioxidant treatment could be more useful at early stages of the disease when there is more intact tissue and it should be started as early as possible.

Acknowledgement

We thank the nursing, medical and supporting staffs of our institution for their careful and dedicated care of the patients.

Funding

This study was supported by Ankara University Scientific Research Project (Project no:20050809007HPD).

References

1. Gabrielli A, Svegliati S, Moroncini G, Pomponio G, Santillo M, et al. (2008) Oxidative stress and the pathogenesis of scleroderma: the Murrell's hypothesis revisited. Semin Immunopathol 30: 329-337.
2. Erre GL, De Muro P, Dellacà P, Fenu P, Cherchi GM, et al. (2008) Iloprost therapy acutely decreases oxidative stress in patients affected by systemic sclerosis. Clin Exp Rheumatol 26: 1095-1098.
3. Gabrielli A, Avvedimento EV, Krieg T (2009) Scleroderma. N Engl J Med 360: 1989-2003.
4. Shah AM, Channon KM (2004) Free radicals and redox signalling in cardiovascular disease. Heart 90: 486-487.
5. Jackson MJ1 (1999) An overview of methods for assessment of free radical activity in biology. Proc Nutr Soc 58: 1001-1006.
6. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18: 872-879.
7. McCord JM1 (2000) The evolution of free radicals and oxidative stress. Am J Med 108: 652-659.
8. Iizuka H, Koizumi H, Kamigaki K, Aoyagi T, Miura Y (1981) Two forms of adenosine deaminase in pig epidermis. J Dermatol 8: 91-95.
9. Solans R, Motta C, Sola R, et al. (2000) Abnormalities of erythrocyte membrane fluidity, lipid composition and lipid peroxidation in systemic sclerosis: evidence of free radical mediated-injury. Arthritis Rheum; 43: 894-900.
10. Volpe A, Biasi D, Caramaschi P, Mantovani W, Bambara LM, et al. (2006) Levels of F2-isoprostanes in systemic sclerosis: correlation with clinical features. Rheumatology (Oxford) 45: 314-320.
11. Herrick AL, Matucci Cerinic M (2001) The emerging problem of oxidative stress and the role of antioxidants in systemic sclerosis. Clin Exp Rheumatol 19: 4-8.
12. Simonini G, Pignone A, Generini S, Falcini F, Cerinic MM (2000) Emerging potentials for an antioxidant therapy as a new approach to the treatment of systemic sclerosis. Toxicology 155: 1-15.
13. Sambo P, Baroni SS, Luchetti M, et al. (2001) Oxidative stress in scleroderma. Meintenance of scleroderma fibroblast phenotype by the constitutive up-regulation of reactive oxygen species generation through the NADPH oxidase pathway. Arhritis Rheum 44: 2653-2664.
14. Tikly M, Marshall SE, Haldar NA, Gulumian M, Wordsworth P, et al. (2004) Oxygen free radical scavenger enzyme polymorphisms in systemic sclerosis. Free Radic Biol Med 36: 1403-1407.
15. Emerit I, Filipe P, Meunier P, et al. (1997) Clastogenic activity in plasma of scleroderma patients: a biomarker of oxidative stress. Dermatology 194: 140-146.
16. Ogawa F, Shimizu K, Muroi E, Hara T, Hasegawa M, et al. (2006) Serum levels of 8-isoprostane, a marker of oxidative stress, are elevated in patients with systemic sclerosis. Rheumatology (Oxford) 45: 815-818.
17. Shimizu K, Ogawa F, Akiyama Y, Muroi E, Yoshizaki A, et al. (2008) Increased serum levels of N(epsilon)-(hexanoyl)lysine, a new marker of oxidative stress, in systemic sclerosis. J Rheumatol 35: 2214-2219.
18. Ogawa F, Shimizu K, Hara T, Muroi E, Hasegawa M, et al. (2008) Serum levels of heat shock protein 70, a biomarker of cellular stress, are elevated in patients with systemic sclerosis: association with fibrosis and vascular damage. Clin Exp Rheumatol 26: 659-662.
19. Iwata Y, Ogawa F, Komura K, et al. (2007) Autoantibody against peroxiredoxin I, an antioxidant enzyme, in patients with systemic sclerosis: possible association with oxidative stress. Rheumatology 46: 790-795.
20. Stein CM, Tanner SB, Awad JA, Roberts LJ 2nd, Morrow JD (1996) Evidence of free radical-mediated injury (isoprostane overproduction) in scleroderma. Arthritis Rheum 39: 1146-1150.
21. Simonini G, Cerinic MM, Generini S, Zoppi M, Anichini M, et al. (1999) Oxidative stress in Systemic Sclerosis. Mol Cell Biochem 196: 85-91.
22. Cracowski JL, Marpeau C, Carpentier PH, Imbert B, Hunt M, et al. (2001) Enhanced in vivo lipid peroxidation in scleroderma spectrum disorders. Arthritis Rheum 44: 1143-1148.
23. Cracowski JL, Carpentier PH, Imbert B, Cachot S, Stanke-Labesque F, et al. (2002) Increased urinary F2-isoprostanes in systemic sclerosis, but not in primary Raynaud's phenomenon: effect of cold exposure. Arthritis Rheum 46: 1319-1323.
24. Luczynska M, Szkudlarek U, Dziankowska-Bartkowiak B, Waszczykowska E, Kasielski M, et al. (2005) Elevated whole blood chemiluminescence in patients with systemic sclerosis. Clin Exp Rheumatol 23: 173-179.
25. Devrim E, Erten S, Erguder IB, Namuslu M, Turgay M, et al. (2008) Malondialdehyde and nitric oxide levels in erythrocytes from patients with systemic sclerosis. Med Princ Pract 17: 349-350.
26. Cope KA, Solga SF, Hummers LK, Wigley FM, Diehl AM, et al. (2006) Abnormal exhaled ethane concentrations in scleroderma. Biomarkers 11: 70-84.
27. Musellim B, Ikitimur H, Uzun H, Ongen G (2006) The oxidant-antioxidant balance in systemic sclerosis cases with interstitial lung involvement. Rheumatol Int 27: 163-167.
28. Rottoli P, Magi B, Cianti R, et al. (2005) Carbonylated proteins in bronchoalveolar lavage of patients with sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis. Proteomics 5: 2612-2618.
29. Ioannidis JP, Vlachoyiannopoulos PG, Haidich AB, Medsger TA Jr, Lucas M, et al. (2005) Mortality in systemic sclerosis: an international meta-analysis of individual patient data. Am J Med 118: 2-10.

30. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee (1980) Preliminary criteria for the classification of systemic sclerosis(scleroderma). Arthritis Rheum 23: 581-590.
31. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, et al. (1988) Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 15: 202-205.
32. Medsger TA Jr, Steen VD. Classification, prognosis. 51-79. In: Systemic sclerosis. Clements PJ, Furst DE (Eds). Baltimore, MD: Williams&Wilkins, 1996.
33. Medsger TA Jr, Bombardieri S, Czirjak L, Scorza R, Della Rossa A, et al. (2003) Assessment of disease severity and prognosis. Clin Exp Rheumatol 21: S42-46.
34. DAHLE LK, HILL EG, HOLMAN RT (1962) The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys 98: 253-261.
35. Hashimoto S (1974) A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. Anal Biochem 62: 426-435.
36. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158-169.
37. Aebi H (1974) Catalase 673-7. In: Methods of enzymatic analysis. Bergmeyer HU, (Ed). New York and London; Verlag Chemie Weinheim Academic Press, 1974.
38. Durak I, Canbolat O, Kavutçu M, Oztürk HS, Yurtarslani Z (1996) Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal 10: 17-20.
39. Giusti G (1974) Adenosine deaminase. 1092-1099. In: Methods of enzymatic analysis. Bergmeyer HU, (Ed). New York and London; Verlag Chemie Weinheim Academic Press, 1974.
40. Murrell DF1 (1993) A radical proposal for the pathogenesis of scleroderma. J Am Acad Dermatol 28: 78-85.
41. Montuschi P, Ciabattoni G, Paredi P, Pantelidis P, du Bois RM, et al. (1998) 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. Am J Respir Crit Care Med 158: 1524-1527.
42. Cracowski JL, Cracowski C, Bessard G, Pepin JL, Bessard J, et al. (2001) Increased lipid peroxidation in patients with pulmonary hypertension. Am J Respir Crit Care Med 164: 1038-1042.
43. Bowers R, Cool C, Murphy RC, Tuder RM, Hopken MW, et al. (2004) Oxidative stress in severe pulmonary hypertension. Am J Respir Crit Care Med 169: 764-769.
44. Morita A, Minami H, Sakakibara N, Sato K, Tsuji T (1996) Elevated plasma superoxide dismutase activity in patients with systemic sclerosis. J Dermatol Sci 11: 196-201.
45. Denton CP, Bunce TD, Dorado MB, Roberts Z, Wilson H, et al. (1999) Probucol improves symptoms and reduces lipoprotein oxidation susceptibility in patients with Raynaud's phenomenon. Rheumatology (Oxford) 38: 309-315.
46. Allanore Y, Borderie D, Lemaréchal H, Ekindjian OG, Kahan A (2004) Acute and sustained effects of dihydropyridine-type calcium channel antagonists on oxidative stress in systemic sclerosis. Am J Med 116: 595-600.
47. Allanore Y, Borderie D, Périanin A, Lemaréchal H, Ekindjian OG, et al. (2005) Nifedipine protects against overproduction of superoxide anion by monocytes from patients with systemic sclerosis. Arthritis Res Ther 7: R93-100.