

Plasma paraoxonase activity in patients with systemic sclerosis

İ.B. Ergüder, Şükran Erten*, Erdinç Devrim, Murat Turgay* & İlker Durak

Ankara University Faculty of Medicine, Departments of Biochemistry & *Clinical Immunology & Rheumatology, Ankara-Turkey

Received July 20, 2007

Background & objectives: Systemic sclerosis (SSc) is a connective tissue disease characterized vascular damage and fibrosis. The aim of this study was to investigate the possible relation between systemic sclerosis and paraoxonase which is an antioxidant enzyme on the HDL.

Methods: Twenty nine patients with SSc and 16 healthy subjects (control group) participated in the study. Plasma cholesterol levels, anti-centromere antibody (ACA) levels and paraoxonase (PON) activities were measured.

Results: Lower level of high-density lipoprotein (HDL) cholesterol was observed in ACA negative SSc patients than in controls. Paraoxonase activity in ACA positive patients was however found to increase relative to control and ACA negative patient groups.

Interpretation & conclusions: Our findings suggested that low HDL level in ACA negative SSc patients might be one of the factors leading to some vascular problems, and increased PON activity in ACA positive SSc group might have some role in the limitation of cutaneous sclerotic process observed in these patients. However, these preliminary findings need to be confirmed with a larger sample.

Key words Anti-centromere antibody - paraoxonase activity - systemic sclerosis

Systemic sclerosis (SSc) is an autoimmune disease of the connective tissue characterized by widespread vascular lesions and progressive fibrosis of the skin, blood vessel and many internal organs such as the kidneys, lungs, heart, gastrointestinal, and musculoskeletal systems. There are two main groups of the disease: the limited cutaneous systemic sclerosis (lSSc) which is expressed as limited skin disease (CREST- Calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia) with high prevalence of anti centromere antibody (ACA), and the diffuse systemic sclerosis (dSSc) which has high prevalence of anti topoisomerase antibodies I (anti-topo I), and worse prognosis¹.

Since vasospasm causes frequent episodes of ischaemia-reperfusion injury and free radical-mediated endothelial dysfunction in SSc, patients with SSc have increased atherosclerotic risk². In these patients, vascular diseases such as Raynaud's phenomenon, pulmonary hypertension (PHT), and atherosclerosis are more common³. Systemic sclerosis is associated with several autoantibodies such as anti-nuclear antibodies (ANA), ACA, anti-Scl-70 (anti-topoisomerase I) antibodies, anti-nucleolar antibodies (ANoA), etc. These autoantibodies are helpful to diagnose patients with SSc and to determine their prognosis. ACA and anti-Scl-70 antibodies are particularly useful for distinguishing patients with SSc from healthy subjects and from patients

with other connective tissue diseases. The presence of ACA is closely associated with CREST and can also distinguish CREST from patients with other variants of SSc, from patients with other connective tissue diseases and from patients with primary Raynaud's phenomenon. The ACA positive patients with SSc generally have a better prognosis than those with SSc-associated other autoantibodies⁴. Additionally, ACA are associated with certain cutaneous and cardiopulmonary manifestations⁵.

Paraoxonase-1 (PON1) enzyme which is located on HDL, is capable of hydrolyzing oxidized low density lipoprotein (ox-LDL). PON1 also protects HDL from oxidation⁶. Due to this antioxidant property of PON1, HDL has antioxidant activity and plays protective role against atherogenesis⁶⁻¹⁰.

This study was undertaken to investigate PON1 activity in plasma samples from patients with ACA positive and negative SSc, and its possible relation with prognosis of the disease including in the limitation of cutaneous sclerotic process.

Material & Methods

The study was performed in the Department of Biochemistry, Ankara University, Faculty of Medicine, Ankara, Turkey. The patients were enrolled from the Clinics of Clinical Immunology & Rheumatology, Ankara University İbn-i Sina Hospital, Turkey. The study protocol was approved by the Ethics Committee of Ankara University, Faculty of Medicine. The samples were collected during the period of September 2005 to February 2006. During this period, 29 patients with SSc (25 women and 4 men; 51.7 ± 12.4 yr mean \pm SD) and 16 healthy volunteers participated as the control group (14 women and 2 men; 55.3 ± 7.9 yr in the study. The chemicals used in study were procured from Sigma, USA. Approximately 10 ml fasting blood specimens were obtained from the patients and healthy subjects into anticoagulated tubes (with EDTA). Cholesterol levels, ACA level and PON activity were measured in plasma. The patients with SSc were divided into two subgroups according to their ACA positivity. In the activity measurement of PON enzyme, phenylacetate was used as substrate¹¹. Enzymatic colorimetric method (Olympus System Reagent, Hamburg, Germany) was used for the measurements of serum TC, LDL-C, HDL-C and TG¹²⁻¹⁵. Anti-centromere antibody was measured with immunoblotting method¹⁵.

One way ANOVA and post hoc LSD test was used for statistical evaluation.

Results & Discussion

HDL cholesterol level was found to be significantly decreased ($P < 0.05$) in the ACA negative SSc patients compared to control group (Table). Paraoxonase activity in ACA positive SSc patients was found to be significantly higher ($P < 0.05$) than those of the control and ACA negative SSc patient groups.

Vascular dysfunction plays an important role in the aetiopathogenesis of SSc, which is known as a microvascular disease⁶. Increased free radical injury, elevated lipoprotein[a] and C-reactive protein (CRP) have been proposed to be involved in the aetiology of vascular pathology^{17,18}. Borba *et al*⁶ showed that patients with limited cutaneous systemic sclerosis, particularly ACA positive ones, have an atherosclerotic lipid profile such as low HDL levels⁶. It has been reported that patients with connective tissue diseases have significantly higher triglyceride, lower HDL cholesterol levels compared with normal subjects¹⁸. However, underlying mechanism(s) of this susceptibility to atherosclerotic progress is not fully understood yet. Presence of atherosclerosis in SSc have been reported by echocardiographic and angiographic examinations and SSc was shown to be associated with intracardiac disorders and elevated atherogenic index^{19,20}. It was also reported that SSc was not associated with clinical atherosclerotic findings²¹. In another study performed by Bruckdorfer *et al*²² LDL levels were shown to be within the reference interval for most of the patients with SSc. However, findings of this study also suggested that free radicals might play a role in the pathology of SSc, and blood LDL fraction from patients with SSc has increased susceptibility to

Table. PON activity and cholesterol levels in SSc patients and controls

	Control group (n=16)	SSc ACA+ve group (n=5)	SSc ACA-ve group (n=24)
PON (IU/ml)	5.44 \pm 1.57	7.9 \pm 3.1**	5.6 \pm 1.8
HDL-C (mg/dl)	40.1 \pm 7.9	37.0 \pm 5.3	34.3 \pm 7.4†
Total-cholesterol (mg/dl)	199.9 \pm 35.3	185.3 \pm 51.5	172.6 \pm 37.2
LDL-C (mg/dl)	125.6 \pm 13.1	135.5 \pm 20.7	109.5 \pm 31.7
VLDL-C (mg/dl)	27.4 \pm 9.7	31.8 \pm 6.6	23.4 \pm 9.9
TG (mg/dl)	129.4 \pm 44.6	136.8 \pm 22.2	116.9 \pm 49.9

Values are given as mean \pm SD; * $P < 0.05$ compared to control; ** $P < 0.05$ compared to ACA -ve group; † $P < 0.05$ compared to control

oxidation compared to healthy controls²². Presence of lipid peroxidation products and antibodies against oxidised low density lipoproteins have been shown in patients affected with SSc²³. ACA is an autoantibody which is produced by patients with SSc and leads to inflammation in various tissues. This may lead to increased antioxidant response in order to decrease oxidant burden.

There are some previous studies on PON activity in Turkish population in literature. One study suggested that the patients with non insulin dependent diabetes mellitus (NIDDM) have lower PON activity than control group²⁴. In another study, Aynacioglu *et al*²⁵ failed to show an association between Gln192Arg polymorphism of PON and risk to develop coronary artery disease (CAD) in Turkish patients, however, they suggested that PON1 may be one of the genes that has been related to the pathogenesis of cardiovascular disease.

Our data suggested that low HDL level in ACA negative SSc patients might be one of the factors leading to some vascular problems in these patients, and increased PON activity in ACA positive SSc group might have some beneficial effect in limiting the cutaneous sclerotic process. Since PON1 is an ester hydrolase entirely bound to HDL, it limits the accumulation of lipid oxidation products in low density lipoproteins (LDL) and prevents the transformation of LDL into atherogenic particles²⁵⁻²⁸.

This is perhaps the first study indicating a possible relation between PON activity and systemic sclerosis. Although small sample size is the limitation of the present study, our findings have importance to elucidate possible role of PON in systemic sclerosis and in the limitation of cutaneous sclerotic process.

Acknowledgment

Authors thank Dr Yasemin Yavuz Genç for statistical evaluation.

References

- Vlachoyiannopoulos PG, Dafni UG, Pakas I, Spyropoulou-Vlachou M, Stavropoulos-Giokas C, Moutsopoulos HM. Systemic scleroderma in Greece: low mortality and strong linkage with HLA-DRB1*1104 allele. *Ann Rheum Dis* 2000; 59 : 359-67.
- LeRoy EC. Systemic sclerosis. A vascular perspective. *Rheum Dis Clin North Am* 1996; 22 : 675-94.
- Ho M, Veale D, Eastmond C, Nuki G, Belch J. Macrovascular disease and systemic sclerosis. *Ann Rheum Dis* 2000; 59 : 39-43.
- Ho KT, Reveille JD. The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther* 2003; 5 : 80-93.
- Hesselstrand R, Scheja A, Shen GQ, Wiik A, Akesson A. The association of antinuclear antibodies with organ involvement and survival in systemic sclerosis. *Rheumatology (Oxford)* 2003; 42 : 534-40.
- Borba EF, Borges CT, Bonfa E. Lipoprotein profile in limited systemic sclerosis. *Rheumatol Int* 2005; 25 : 379-83.
- Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet* 2001; 68 : 1428-36.
- von Eckardstein A, Schmidem K, Hövels A, Gulbahce E, Schuler-Luttmann S, Elbers J, *et al*. Lowering of HDL cholesterol in post-menopausal women by tibolone is not associated with changes in cholesterol efflux capacity or paraoxonase activity. *Atherosclerosis* 2001; 159 : 433-9.
- Mackness B, McElduff P, Mackness MI. The paraoxonase-2-310 polymorphism is associated with the presence of microvascular complications in diabetes mellitus. *J Intern Med* 2005; 258 : 363-8.
- Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001; 21 : 473-80.
- Kitchen BJ, Masters CJ, Winzor DJ. Effects of lipid removal on the molecular size and kinetic properties of bovine plasma arylesterase. *Biochem J* 1973; 135 : 93-9.
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20 : 470-5.
- Miki Y. A homogeneous assay for the selective measurement of LDL-cholesterol in serum. Enzymatic selective protection method. *Clin Lab* 1999; 45 : 398-401.
- Laboratory measurement of lipid, lipoproteins and apolipoproteins*. Rifai N, Warnick GR, editors. Washington, DC, USA: AACC Pres; 1994.
- Katano K, Kawano M, Koni I, Sugai S, Muro Y. Clinical and laboratory features of anticentromere antibody positive primary Sjogren's syndrome. *J Rheumatol* 2001; 28 : 2238-44.
- Murrell DF. A radical proposal for the pathogenesis of scleroderma. *J Am Acad Dermatol* 1993; 28 : 78-85.
- Lippi G, Caramaschi P, Montagnana M, Salvagno GL, Volpe A, Guidi G. Lipoprotein[a] and the lipid profile in patients with systemic sclerosis. *Clin Chim Acta* 2006; 364 : 345-8.
- Matteucci E, Migliorini P, Bertoni C, Dolcher MP, Marchini B, Giampietro O. An early diagnosis of kidney involvement in immunologically-mediated multisystem diseases. *Clin Rheumatol* 1996; 15 : 20-4.
- Kozlova LK, Tamgina TF, Nuzhdina TV, Antonenko TV, Alekhina EM. Echocardiographic functional assessment of the heart and lipid metabolism in patients with systemic scleroderma and systemic lupus erythematosus. *Ter Arkh* 2001; 73 : 33-6.

20. Dick EA, Aviv R, Francis I, Hamilton G, Baker D, Black C, *et al.* Catheter angiography and angioplasty in patients with scleroderma. *Br J Radiol* 2001; 74 : 1091-6.
21. Zakopoulos NA, Kotsis VT, Gialafos EJ, Papamichael CM, Pitiriga VC, Mitsibounas DN, *et al.* Systemic sclerosis is not associated with clinical or ambulatory blood pressure. *Clin Exp Rheumatol* 2003; 21 : 199-204.
22. Bruckdorfer KR, Hillary JB, Bunce T, Vancheeswaran R, Black CM. Increased susceptibility to oxidation of low-density lipoproteins isolated from patients with systemic sclerosis. *Arthritis Rheum* 1995; 38 : 1060-7.
23. Simonini G, Cerinic MM, Generini S, Zoppi M, Anichini M, Cesaretti C, *et al.* Oxidative stress in systemic sclerosis. *Mol Cell Biochem* 1999; 196 : 85-91.
24. Agachan B, Yilmaz H, Karaali Z, Isbir T. Paraoxonase 55 and 192 polymorphism and its relationship to serum paraoxonase activity and serum lipids in Turkish patients with non-insulin dependent diabetes mellitus. *Cell Biochem Funct* 2004; 22 : 163-8.
25. Aynacioglu AS, Kepekci Y. The human paraoxonase Gln-Arg192 (Q/R) polymorphism in turkish patients with coronary artery disease. *Int J Cardiol* 2000; 74 : 33-7.
26. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett* 1991; 286 : 152-4.
27. La Du BN. Human serum paraoxonase/ arylesterase. In: Kalow W, editor. *Pharmacogenetics of drug metabolism*. New York: Pergamon Press; 1992. p. 51-92.
28. Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, *et al.* Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995; 96 : 2882-91.

Reprint requests: Dr B. Imge Ergüder, Ankara Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı Dekanlık Binası
Sıhhiye 06100 / Ankara - Turkey
e-mail: imgeerguder@yahoo.com