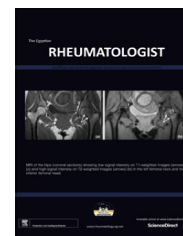




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ORIGINAL ARTICLE

# Study of anti-apolipoprotein A-I antibodies and paraoxonase 1 activity in systemic lupus erythematosus patients; correlation with disease activity and damage indices



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## KEYWORDS

Systemic lupus erythematosus (SLE);  
Anti-apolipoprotein A-I antibody (anti-Apo A-I);  
Paraoxonase 1 (PON1)

**Abstract** *Introduction:* Systemic lupus erythematosus (SLE) patients have an increased risk of atherosclerosis. Identification of at-risk patients and the pathogenesis of atherosclerosis in SLE remain elusive. Paraoxonase 1 (PON1) and anti-apolipoprotein A-I antibody (anti-Apo A-I) appear to have a potential role in premature atherosclerosis in SLE.

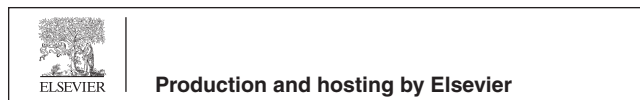
*Aim of the work:* To assess two novel risk factors of atherosclerosis in SLE patients; PON1 activity, and anti-Apo A-I antibody levels, in order to elucidate any possible correlation between both of them, and to demonstrate their relations to disease activity as well as disease related damage.

*Patients and methods:* Forty SLE female patients and 40 apparently healthy volunteers were included in this study. Anti-Apo A-I antibody levels and PON1 activity levels were assessed. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and Systemic Lupus International Collaboration Clinics (SLICC)/American College of Rheumatology (ACR) damage index were performed to all patients.

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**Results:** Compared with controls, SLE patients showed significantly lower PON1 activity and significantly higher titers of anti Apo A-I. Anti-Apo A-I antibody titers correlated inversely with PON1 activity. Elevated titers of anti-Apo A-I antibody and reduced PON1 activity were related to increased SLEDAI and (SLICC/ACR) damage index scores.

**Conclusion:** There is a decreased PON1 activity and formation of anti-Apo A-I antibodies in SLE patients and both of them correlated with disease activity as well as disease-related damage. PON1 activity and anti-Apo A-I antibodies might be involved in the pathogenesis of premature atherosclerosis in SLE patients.

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## 1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disorder that primarily affects women and can involve virtually any organ in the body [1]. It is characterized by the presence of autoantibodies against multiple self antigens and immune complexes that target multiple organ systems [2].

With the increased life expectancy among SLE patients, accelerated atherosclerosis and subsequent cardiovascular disease (CVD) have emerged as a significant threat that significantly contributes to morbidity and mortality [3].

In recent years, there has been a growing interest in understanding the pathogenesis of accelerated atherosclerosis in SLE [4]. Traditional risk factors fail to account fully for the excess CVD events in SLE patients. Therefore, it has been suggested that patients possess additional SLE-related risk factors [5].

Epidemiological studies have demonstrated a strong inverse relationship between high density lipoprotein (HDL) level and risk for CVD [6]. HDL has a wide range of functions including: reverse cholesterol transport, antioxidant, anti-inflammatory, anti-thrombotic and anti-atherogenic activity [7]. The protective functions of HDL could be partly explained by its constituents mainly, apolipoprotein A-I (Apo A-I) and paraoxonase (PON1) [8].

Apo A-I is the major protein component of HDL and is widely considered to be responsible for the anti-atherogenic and anti-thrombotic effects of HDL by promoting cellular cholesterol efflux and exerting anti-oxidative and anti-inflammatory effects [8]. Apo A-I exerts anti-oxidant properties by stabilizing PON1 [9]. PON1 is an antioxidant enzyme attached to HDL. PON1 decreases systemic oxidative stress and is associated with a lower incidence of CVD [10].

There is an emerging evidence of the presence of anti-Apo A-I antibodies and the reduction in the plasma levels of PON1 in SLE patients, thus interfering with the protective functions of HDL favoring atherogenesis [11].

The aim of the present study was to assess two novel risk factors of atherosclerosis in SLE patients; PON1 activity, anti-Apo A-I antibody levels, to find any possible correlation between both of them, and to demonstrate their relations to disease activity as well as disease related damage.

## 2. Patients and methods

### 2.1. Patients

Eighty participants were included in this study; they were divided into two groups:

#### 2.1.2. Group (A): patient group

Forty SLE premenopausal female patients were diagnosed according to the ACR revised criteria of SLE [12]. Their age ranged between 18 and 46 years and the SLE disease duration extended between 0.5 and 15 years. These patients were recruited from Rheumatology and Rehabilitation outpatient clinic and department of Kasr El-Aini hospitals (Cairo University). SLE patients with parameters known to influence the PON1 activity or induce premature atherosclerosis were excluded namely, smoking, diabetes mellitus, chronic renal failure or nephrotic syndrome, and antiphospholipid syndrome. Patients on lipid lowering drugs, known cases of primary dyslipidemia and hypothyroidism and family history of CVD were also excluded.

#### 2.1.3. Group (B): control group

Forty apparently healthy, age – matched female volunteers served as the control group. Their age ranged between 18 and 47 years.

An informed consent was obtained from all participants in the study, and the study was approved by the Institutional Review Board (IRB) of faculty of medicine, Cairo University.

### 2.2. Methods

All participants were subjected to the following:

- 1. Comprehensive history taking and thorough clinical examination;** general, cardiopulmonary, abdominal, neurological, and musculoskeletal system.
- 2. Routine laboratory investigations** (CBC, ESR, liver and kidney functions, and urine analysis, in addition to estimation of total albumin in 24 h urine), lipid profile (triglycerides and total serum cholesterol, HDL, and LDL concentration), immunological assays (ANA, anti-dsDNA antibodies, anticardiolipin antibodies, and lupus anticoagulants), and serum complement levels (C3 and C4).
- 3. Determination of anti-Apo A-I antibody levels in the plasma;** 96-well plates (PolySorp) were half-coated for 1 h at 37 °C with 10 g/ml human Apo A-I (Sigma–Aldrich) in 70% ethanol. Blocking was performed using phosphate buffered saline containing 1% albumin from bovine serum for 1 h at 37 °C. Hundred microliters of the samples (1:300 dilutions in blocking agent) and positive control were added to duplicate wells in both halves of the plate and kept for 1 h at 37 °C. After washing, alkaline phosphatase-conjugated anti-human IgG (1:1000 in the blocking agent) was added for 1 h. *p*-Nitrophenyl phosphate (1:5000 in

bicarbonate buffer, pH 9.8) was added and incubated at 37 °C for color development and the absorbance read at 405 nm after 1 h. All assays were validated by the inclusion of internal quality control samples of known activity. Results were expressed as the percentage of the positive control present in each plate after subtraction from the background in the uncoated half of the plate. Inter-/intra-plate coefficients of variation were < 10% [11].

4. **Determination of paraoxonase 1 activity;** serum paraoxonase 1/arylesterase (the activity toward phenyl acetate) was determined spectrophotometrically at 270 nm with phenyl acetate used as the substrate. The assay mixture included 1.0 mmol/L of phenyl acetate and 0.9 mmol/L CaCl<sub>2</sub> in 20 mmol/L Tris HCl, pH 8.0, at 25 °C. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The E<sub>270</sub> for the reaction is 1310 mol/L<sup>-1</sup> cm<sup>-1</sup>. The results are expressed in U/ml; 1 U hydrolyzes 1 μmol of phenyl acetate/minute [13].
5. **Radiological investigations;** chest X-ray, X-ray for any affected joint.
6. **Electrocardiography (ECG);** for detection of ischemia, pericardial effusion, pericarditis, and myocarditis.
7. **Assessment of disease activity using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI);** SLEDAI is a scale designed specifically to assess the disease activity in SLE patients. It measures potentially reversible manifestations of the underlying inflammatory disease process. This disease activity index covers the symptoms and laboratory changes at the time of examination as well as in the previous 10 days before examination. It consists of 24 variables covering nine organ systems (including some serologic tests) scored according to weights derived using multiple regression techniques. The final score comprises the sum of all weighted scores. Grading of disease activity to mild (1–10), moderate (11–20), severe (21–45), and very severe (> 45) [14].
8. **Assessment of disease related damage was done using Systemic Lupus International Collaborative Clinics/American College of Rheumatology (SLEICC/ACR) damage index;** the damage in SLE may be due to SLE itself or due to drug therapy. The index records damage in 12 organs or systems. The change must have been present for at least 6 months and is ascertained clinically or by simple investigation [15].

*Statistical analysis:* Data were statistically described in terms of mean ± standard deviation (±SD). A comparison between cases and control groups was done using Student's *t* test for independent samples. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal variables. *p* Values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS version 15 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA).

### 3. Results

Eighty participants were included in this study; they were divided into two groups; Group (A) included 40 SLE premenopausal female patients diagnosed according to ACR revised criteria of SLE. Their age ranged between 18 and

46 years with a mean of 28.35 ± 7.06 years. The disease duration extended between 0.5 and 15 years with a mean of 4.58 ± 3.36 years. Their body mass index (BMI) ranged between 19.72 and 44.41 kg/m<sup>2</sup> with a mean of 26.19 ± 4.48, where 11 patients had a normal BMI, 4 were underweight, 18 were overweight, 6 were obese and only 1 patient was morbidly obese. As for Group B 40 apparently healthy age – matched females served as the control group. Their age ranged between 18 and 47 with a mean of 28.45 ± 7.53 years. Their BMI ranged between 20.8 and 42.6 kg/m<sup>2</sup> with a mean of 26.38 ± 4.01, where 15 of them had a normal BMI, 19 were overweight, 5 were obese and only 1 volunteer was morbidly obese.

The clinical characteristics of Group (A) patients are listed in Table 1, while the laboratory characteristics of both groups are shown in Table 2.

**Table 1** Clinical characteristics of Systemic lupus erythematosus patients (Group A) (*n* = 40).

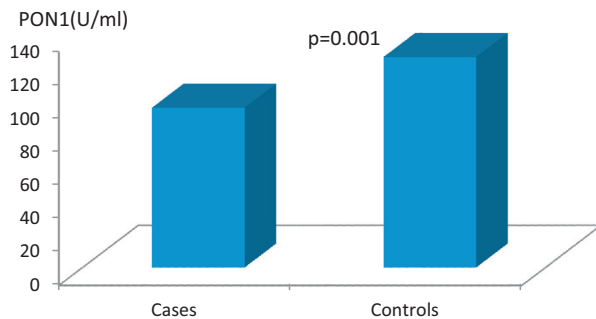
Clinical data	<i>n</i> (%)
Musculoskeletal manifestation	37 (92.5)
Arthralgia	37 (92.5)
Frank arthritis	28 (70)
Avascular necrosis of bone	6 (15)
Tendinitis	4 (10)
Myositis	3 (7.5)
Deformity	1 (2.5)
Mucocutaneous manifestation	32 (80)
Hair fall	23 (57.5)
Oral ulcer	22 (55)
Skin rash	20 (50)
Photosensitivity	18 (45)
Raynaud's phenomenon	7 (17.5)
Scarring alopecia	2 (5)
Constitutional manifestation	30 (75)
Fatigue	24 (60)
Fever	21 (52.5)
Weight loss	9 (22.5)
Nephritis	26 (65)
Serositis	16 (40)
Pleurisy	16 (40)
Pericarditis	2 (5)
Central nervous system manifestation	12 (30)
Psychosis	7 (17.5)
Convulsions	6 (15)
Organic brain syndrome	3 (7.5)
Neuropathy and numbness	3 (7.5)
Lupus headache	1 (2.5)
Hepatomegaly	10 (25)
Vasculitis	7 (17.5)
Skin vasculitis	5 (12.5)
Retinal vasculitis	3 (7.5)
Ocular manifestations	7 (17.5)
Retinal vasculitis	3 (7.5)
Episcleritis	1 (2.5)
Secondary Sjögren's syndrome	4 (10)
Splenomegaly	5 (15)
Lymphadenopathy	3 (7.5)

**Table 2** Laboratory characteristics of studied Groups: SLE patients (Group A) and control (Group B).

Parameter	Group (A): patients		Group (B): controls		p Value
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
Hb (g/dl)	10.7 $\pm$ 2.02	5.9–13.4	12.45 $\pm$ 0.83	10.5–13.8	0.000*
TLC ( $\times 10^3/\text{mm}^3$ )	6.2 $\pm$ 2.36	1.6–11.7	6.95 $\pm$ 1.98	4.1–11.1	NS
Platelets ( $\times 10^3/\text{mm}^3$ )	256.8 $\pm$ 88.62	75–421	263.15 $\pm$ 53.07	172–371	NS
ESR (mm/1sth)	52.5 $\pm$ 24.78	10–120	14.55 $\pm$ 6.04	5–26	0.000*
ALT (U/L)	23.52 $\pm$ 12	7–73	25.45 $\pm$ 12.55	5–65	NS
AST (U/L)	26.18 $\pm$ 11.98	9–69	27.43 $\pm$ 15.36	11–80	NS
Urea (mg/dl)	36.08 $\pm$ 16.35	13–90	31.8 $\pm$ 7.13	13–43	NS
Creatinine (mg/dl)	0.79 $\pm$ 0.37	0.4–2.4	0.73 $\pm$ 0.2	0.5–1.2	NS
TC (mg/dl)	259.08 $\pm$ 68.75	143–376	159 $\pm$ 38.15	73–240	0.000*
TG (mg/dl)	228.23 $\pm$ 83.27	96–428	127.03 $\pm$ 36.02	76–213	0.000*
HDL (mg/dl)	41.88 $\pm$ 6.92	31–60	59.5 $\pm$ 10.5	39–78	0.000*
LDL (mg/dl)	171.53 $\pm$ 54.12	81–265	74.8 $\pm$ 24.77	19–120	0.000*
C <sub>3</sub> (mg/dl)	92.28 $\pm$ 52.54	13.5–185	135.49 $\pm$ 25.42	90.3–185	0.000*
C <sub>4</sub> (mg/dl)	16.73 $\pm$ 12.74	2.5–43	28.52 $\pm$ 8.28	12.1–42	0.000*

Hb: hemoglobin, TLC: total leucocytic count, ESR: erythrocyte sedimentation rate, ALT: alanine transaminase, AST: aspartate transaminase, TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein, C: complement.

\* Significant ( $p < 0.05$ ) NS: nonsignificant.

**Figure 1** Comparison between PON1 activity in Group (A) SLE patients and Group (B) Controls.

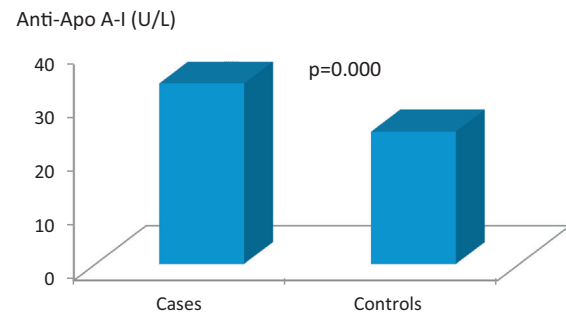
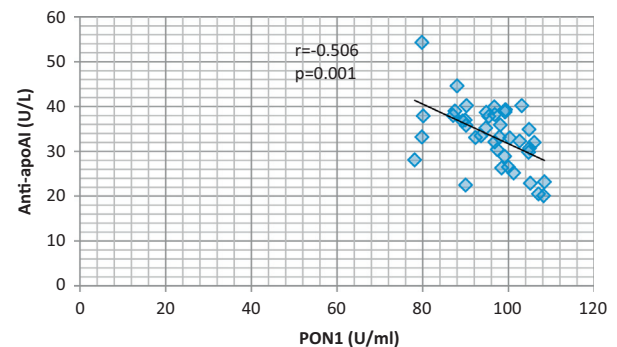
Regarding drug consumption among Group (A) patients, all of them (100%) were on corticosteroids, and in addition 31 (77.5%) were receiving antimalarials, 22 (55%) were on azathioprine, and only 9 patients (22.5%) were on cyclophosphamide therapy.

The serum paraoxonase 1 (PON1) activity levels were  $126.2 \pm 7.79$  U/ml (range 99.8–138.1 U/ml) in Group (B) controls, as compared to  $95.92 \pm 8.11$  U/ml (range 78.1–108.4 U/ml) in group (A) SLE patients, which was significantly lower ( $p = 0.001$ ) (Fig. 1).

The mean plasma anti-Apo A-I antibody level (Anti-Apo A-I) in Group (B) controls was  $24.57 \pm 6.51$  U/L (range 17.5–39.6 U/L), as compared to  $33.5 \pm 6.92$  U/L (range 20.1–54.3 U/L) in Group (A) SLE patients, which was significantly higher ( $p = 0.000$ ) (Fig. 2).

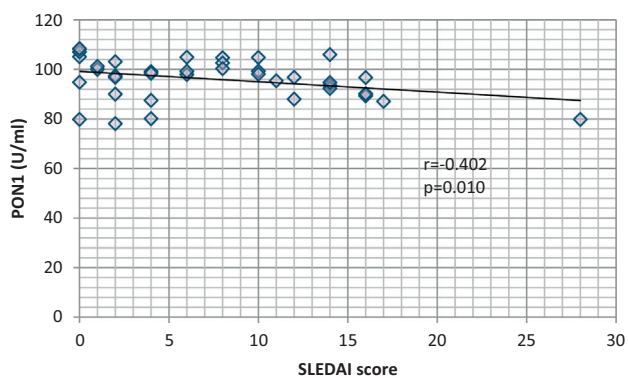
Additionally, a significant negative correlation was established between serum PON1 activity level and plasma anti-ApoA-I level among Group (A) SLE patients ( $r = -0.506$ ,  $p = 0.001$ ) (Fig. 3).

The mean disease activity index (SLEDAI) scores in group (A) SLE patients was  $7.9 \pm 6.67$  (range 0–28), which showed a significant negative correlation with the PON1 activity ( $r = -0.402$ ,  $p = 0.01$ ) (Fig. 4), and a significant positive

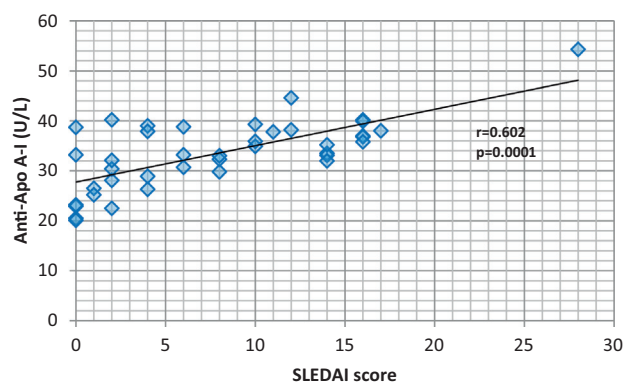
**Figure 2** Comparison between anti-Apo A-I antibody (Anti-Apo A-I) levels in Group (A) SLE patients and Group (B) Controls.**Figure 3** Correlation between the PON1 activity and anti-Apo A-I levels among Group (A) patients.

correlation with the anti-Apo A-I antibody levels ( $r = 0.602$ ,  $p = 0.0001$ ) (Fig. 5).

The damage index among Group (A) SLE patients as expressed by the SL ICC/ACR scores was  $0.95 \pm 0.96$  (range 0–3), where 29 patients showed no damage. Patients with

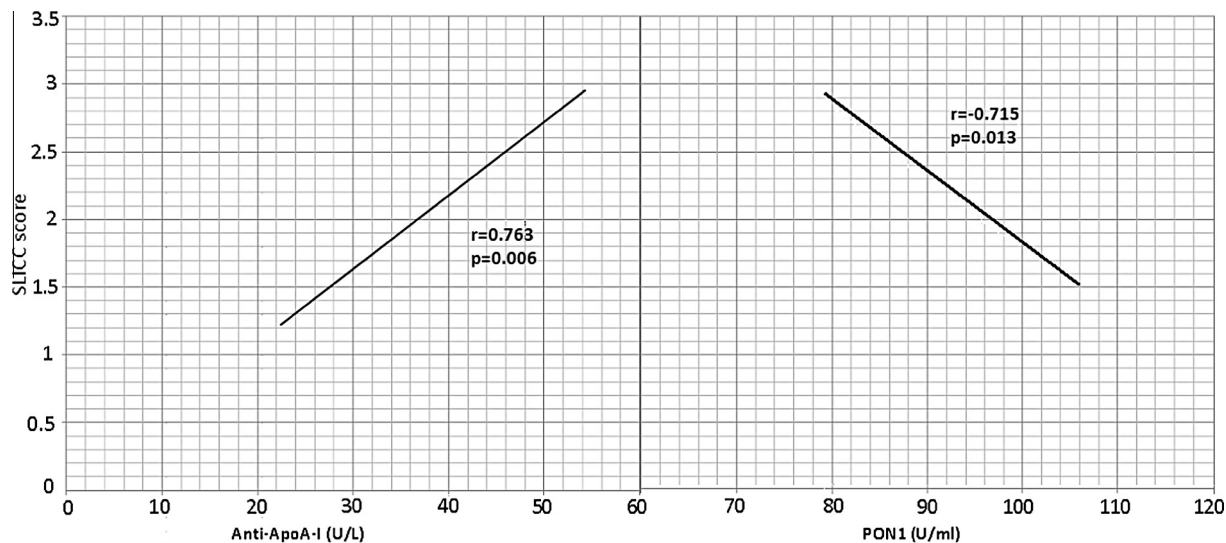


**Figure 4** Correlation between PON1 activity and SLEDAI scores in group (A) SLE patients.



**Figure 5** Correlation between anti-Apo A-I levels and SLEDAI scores in group (A) SLE patients.

scores from 1 to 3 showed a significant negative correlation between SLICC/ACR scores and PON1 activity ( $r = -0.715$ ,  $p = 0.013$ ) and on the contrary a significant positive correlation was elicited with the anti-Apo A-I antibody levels ( $r = 0.763$ ,  $p = 0.006$ ) (Fig. 6).



**Figure 6** Correlation between both PON1 activity and anti-Apo A-I antibody levels with the SLICC scores in SLE patients.

The correlation between the demographic characteristics of group (A) SLE patients (age, disease duration or BMI) did not show any significance.

As for the clinical characteristics, the PON1 activity levels showed a statistically significant difference between the presence and absence of constitutional symptoms ( $p = 0.038$ ), and CNS manifestations ( $p = 0.03$ ).

Regarding laboratory investigations, the anti-Apo A-I levels showed a significant negative correlation with serum levels of both C3 ( $r = -0.316$ ,  $p = 0.047$ ) and C4 ( $r = -0.416$ ,  $p = 0.008$ ).

The therapeutic protocols did not show any significant correlation with the PON1 activity nor anti-Apo A-I levels, regarding drugs, duration of treatment, nor doses.

#### 4. Discussion

Systemic lupus erythematosus is a chronic inflammatory disorder characterized by the production of autoantibodies against a variety of self antigens, generation of immune complexes, and activation of the complement system [16].

Patients with SLE have a significantly increased risk of CVD morbidity, and mortality, particularly related to premature atherosclerosis [17].

Epidemiological studies have consistently shown that plasma HDL was inversely correlated with the incidence of CVD in the general population. This relationship is actually quite complex and involves not only the quantity, but also the quality of HDL. The importance of HDL as a protective molecule against atherogenesis could be partly explained by its constituents, mainly *Apo A-I* and *PON1* [18,19].

Paraoxonase 1 is an enzyme synthesized in the liver and is bound to HDL particles in blood. PON1 appears to contribute to the antioxidant and anti-atherosclerotic capabilities of HDL [10]. However, the exact mechanism of PON1's protective action and its endogenous substrate remain elusive [20].

The objective of the present study was to assess the two novel risk factors of atherosclerosis in SLE patients; PON1 activity, anti-Apo A-I antibody levels, to find any possible

correlation between both of them, and to demonstrate their relations to disease activity as well as disease related damage.

Forty adult SLE premenopausal female patients diagnosed according to the ACR revised criteria of SLE and 40 apparently healthy age and culture matched female controls were included in this study.

Our study revealed statistically significant lower PON1 activity in SLE patients compared to healthy controls. Thus, our patients are prone to atherosclerosis and its complications. There is an increasing epidemiological evidence that the PON1 protects against the development of atherosclerosis and is an independent risk factor for CVD [21]. Our findings agree with those of another study which showed that a decreased PON1 activity was associated with clinical atherothrombotic complications [22]. Furthermore, the atheroprotective function of the PON1 has also been demonstrated in PON1 knock-out mice, which exhibited an accelerated atherosclerosis in contrast to human PON1 transgenic mice where the lesion size was decreased [23].

The decreased PON1 activity in our patients may be a cause, or a consequence of SLE. This issue remains unclear. As a cause, Tripi et al. [24] concluded that a low PON1 activity is independently associated with SLE and certain PON1 polymorphisms are associated with lupus nephritis [24]. On the contrary, a reduced PON1 activity could be a consequence of inflammation as inflammation and proinflammatory cytokines diminish the PON1 activity. In addition, inhibition of PON1 may be a consequence of SLE associated oxidative stress [11,24].

Assessment of the PON1 activity in SLE patients showed conflicting results. Most studies reported a decline in the PON1 activity [11,25,26]. These investigators assessed the PON1 activity by paraoxon as a substrate in their assay. On the other hand, Tripi et al. [24] as well as Kiss et al. [22] used both phenyl acetate and paraoxon as substrates in their assay in the same SLE patients, and both studies found a significant decline in the PON1 activity when assessed by paraoxon [22,24]. However, on assessment of the PON1 activity by phenyl acetate Tripi et al. [24] reported a significant increase in the PON1 activity in their patients [24], whereas, Kiss et al. [22] did not find any significant change in the PON1 activity [22]. In our study we used phenyl acetate as a substrate for assessment of the PON1 activity.

These contrasting results of the PON1 activity levels in SLE may be attributed to the fact that the PON1 activity differs greatly among ethnic groups. Inter-individual variation in the PON1 activity is under a strong genetic influence [27]. In addition, factors including gender, lifestyle factors, and pharmacological agents can also modulate the PON1 activity [28]. On the other hand, the difference in the results of PON1 activity levels in the same patients of Tripi et al. [24] and Kiss et al. [22] may be attributed to genotype variation and polymorphism [22,24]. Polymorphisms can influence enzyme activity i.e. the same polymorphism can generate opposite effects on the PON1 activity depending on the type of substrate used [28].

Using phenyl acetate in our study as a substrate minimizes the role of genotype variation as suggested by Precourt et al. [28]. It is considered as the best substrate in reflecting the antioxidant activity of PON1 [29]. The difference between our results and those of Tripi et al. [24] and Kiss et al. [22] who used phenyl acetate also in their studies appears to be due to the exclusion of the individuals taking statins from our study.

The use of statins has in general been associated with an increase in the PON1 serum activity measures [30].

Regarding the correlation between PON1 activity and SLE disease activity assessed by SLEDAI scores in our study, PON1 activity showed statistically significant negative correlation with such scores. In concordance with our results, Batuca et al. [11] found a significant negative correlation between PON1 activity and British Isles Lupus Assessment Group (BILAG-2004) disease activity index [11].

It has been reported that SLE activity is associated with deterioration of the antioxidant status [11]. Increased oxidative stress inactivates PON1, and a decline in the PON1 activity in turn augments oxidative stress. Therefore, the trio of disease activity, oxidative stress, and PON1 activity forms a vicious circle and this explains the negative correlation between PON1 activity and SLEDAI [30,31].

In contrast to our results Kiss et al. [22] found no correlation between PON1 activity and SLEDAI, which may be attributed to the relatively lower SLEDAI scores of the patients included in their study (2 versus 7.9) [22].

There was a statistically significant negative correlation between SLICC/ACR damage index scores and the PON1 activity in our study. Our data are consistent with a previous report [11].

The reported oxidative stress in SLE can damage macromolecules, including DNA and can exacerbate inflammation leading to tissue damage [32]. This may be an explanation for the statistically significant negative correlation between SLICC/ACR scores and the PON1 activity.

In this study, statistically significant differences were obtained between the PON1 levels in the presence and absence of constitutional and CNS manifestations only. Otherwise, no significant differences were present between the PON1 levels in the presence and absence of other clinical manifestations. To our knowledge, this is the first study to address this relationship between PON1 activity levels and constitutional as well as CNS manifestations in SLE. On the other hand, Tripi et al. [24] found decreased (though non-significant) PON1 activity levels in lupus nephritis patients compared to SLE patients without lupus nephritis [24].

Apolipoprotein A-I is another important component of HDL and it has cardioprotective effects. It plays a major role in cholesterol homeostasis and it exerts both anti-inflammatory and antioxidant properties [8]. Apo A-I exerts its anti-oxidant properties by stabilizing the PON1 enzyme [9]. Deficiency of Apo A-I could lead to deranged processes of immunity [33]. Autoantibodies directed to Apo A-I molecules may inhibit their normal functions [34]. These antibodies may be present in general population but in low titers and they may be related to the vascular and immune aging processes [26].

In this study, there was a statistically significant higher anti-Apo A-I antibody level in SLE patients as compared to controls, as previously reported in many researches [11,26,33,35,36]. However, there is paucity of information about its exact pathophysiological role [34]. Anti-Apo A-I antibody production may be explained by the fact that autoantibody production against multiple self antigens is considered as a hallmark of SLE [37]. Oxidative stress reported in SLE may be a possible mechanism for increased autoantibody production. In autoimmune diseases, oxidative stress is the major event causing structural modifications of proteins with consequent appearance of neopeptides which can become targets of autoimmune reactions,

thus sustaining the inflammatory mechanisms involved in endothelial dysfunction and plaque development [38].

These autoantibodies may lead to Apo A-I dysfunction. Apo A-I normally contributes in the removal of damaged and apoptotic endothelial cells. Thus, anti-Apo A-I antibodies that inhibit these normal functions could predispose to autoimmunity and may play a role in the pathogenesis of SLE. Even if these antibodies arise later, in the development of the disease, and even if the disease is already established, these antibodies may promote a vicious cycle perpetuating the pathological process underlying the disease [33,34].

Apolipoprotein A-I antibodies can affect atherosclerosis and thrombosis independent of autoimmunity [34]. It could represent an emerging CVD risk factor and an independent predictor of major CVD events [39]. The presence of these antibodies in our patients might have exposed them to accelerated atherosclerosis as they lose their atheroprotection mediated by Apo A-I. These antibodies can also affect the normal atheroprotective function of HDL, and the formed dysfunctional HDLs are proinflammatory [34].

Our study revealed statistically significant lower C3 and C4 levels in SLE patients compared to healthy controls. However, the mean values of C3 and C4 in the SLE patients and controls were within the normal range. Thus, there may be a mild degree of complement activation.

A negative correlation was obtained between anti-Apo A-I antibody with C3 and C4 levels. Circulating immune complexes (antigen-antibody interaction) have been correlated with complement activation and autoantibody profiles in SLE [40]. This may be a potential explanation for the activation and consumption of C3 and C4 associated with the increase in production of autoantibodies against Apo A-I in our patients.

There was a significant positive correlation between anti-Apo A-I antibody and SLE disease activity was assessed by SLEDAI scores in our patients. In SLE, levels of certain antibodies such as anti-dsDNA frequently correlate with the disease activity [36].

Similarly, a significant positive correlation was reported between anti-Apo A-I antibody levels and SLE disease activity assessed by other different scoring systems such as BILAG [11,26], and the European Consensus Lupus Activity Measurement (ECLAM) [33].

Furthermore, O'Neill et al. [36] assessed disease activity by BILAG over a 2-year period. They reported that levels of anti-Apo A-I antibody were significantly higher in patients with a history of persistently high disease activity than in those with persistently quiescent disease [36].

In contrast to our results, Shoenfeld et al. [33] did not report any correlation between anti-Apo A-I antibody levels and SLEDAI scores among Italian patients [33].

Our data showed a statistically significant positive correlation between SLICC/ACR scores and anti-Apo A-I antibody levels, as confirmed by previous study [11]. This can be explained by the formation of antibody-antigen complexes that can attach to tissues, fix complement, and cause its damage [34].

Compared to controls, our patients have dyslipidemia in the form of reduced levels of HDL and elevated levels of TC, TG, and LDL, which appears to be related to corticosteroid therapy in our patients. This pattern of dyslipidemia is associated with adverse CVD risk in the general population

[41,42]. Two different patterns of dyslipidemia have been described in SLE. One pattern, referred to as "active SLE profile", is characterized by decreased HDL, and elevated LDL and TG. The second pattern resembles the first one but with increased levels of TC, and is seen with the high dose of steroid therapy [43].

Based on these studies, our patients appear to be in a pro-atherogenic state. Traditional risk factors involved in the pathogenesis of atherosclerosis in our patients include dyslipidemia, corticosteroid administration and hypertension. SLE-related risk factors in our patients include: immune system involvement (anti-Apo A-I antibody production), and oxidative stress (reduced PON1 activity) [18].

Increasing evidence has highlighted the role of oxidative stress in both SLE as well as atherosclerotic CVD. Although, the molecular bases of the interaction between increased oxidative stress, SLE and atherosclerosis remain unclear [31,44], our study may provide a plausible explanation for these clues.

Paraoxonase 1 (PON1) physiologically plays an important role in decreasing oxidative stress and atherosclerosis risk, as it accounts for most of the capacity of HDL to prevent oxidation of LDL [21]. So, the reduced PON1 activity in our patients might have a deleterious effect on the protective function of HDL. SLE is associated with intense antibody production and a possible mechanism for the reduced antioxidant PON1 activity may be the autoantibody production against Apo A-I present in our patients [45]. The statistically significant negative correlation between PON1 and anti-Apo A-I antibody in the present study together with other research groups highlighted this concept [11,26].

Our results suggest the presence of a vicious circuit between PON1 activity and anti-Apo A-I antibody. Reduced PON1 activity affects HDL functions and this enhances the oxidative stress. Oxidative stress leads to the formation of new antigens which in turn enhances the humoral response leading to the production of autoantibodies including anti-Apo A-I antibodies. Anti-Apo A-I antibodies target PON1 reducing its activity [21,45]. Such a vicious circuit with its components maintains oxidative stress and subsequent SLE-related pro-atherogenic state.

Diminished HDL levels have been recognized as an independent risk factor for CVD. However, there is an increasing evidence that HDL quality may be as important as its quantity. The concept of "dysfunctional HDL" or "proinflammatory HDL" is an emerging issue. Dysfunctional HDL converts a positive force protecting arteries to a negative one, enhancing atherogenesis [46]. Among the factors that convert HDL into "dysfunctional HDL" is the decrease of the major antioxidant enzyme PON1. Another major structural and functional component of HDL is Apo A-I. Autoantibodies against Apo A-I may lead to Apo A-I dysfunction with secondary HDL dysfunction [34,47].

High density lipoprotein (HDL) may be affected in our SLE patients in the same manner. The two main components that account for most of the protective functions of HDL are affected. PON1 activity is diminished and Apo A-I are targeted by an anti-Apo A-I antibodies. So, our patients appear to have dysfunctional HDL which represents another pro-atherogenic factor in our SLE patients [47].

Finally, over the next few years, the two SLE related risk factors of atherosclerosis in this study anti-Apo A-I antibodies and/or the PON1 activity may be recommended as a routine

strategy to stratify SLE patients for risk of accelerated atherosclerosis.

In conclusion, there is a decreased PON1 activity and formation of anti-Apo A-I antibodies in patients with SLE. Both of them may be involved in the proatherogenic state noticed in these patients. SLE-disease activity assessed by SLEDAI and SLE disease related organ damage assessed by SLICC/ACR damage index are negatively correlated with the PON1 activity and positively correlated with anti-Apo A-I antibodies.

#### Conflict of interest

None.

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