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Carotid intima-media thickness and paraoxonase activity in patients with ankylosing spondylitis

Abstract

Purpose: The risk of atherosclerosis is increased in several rheumatological disorders, but any such risk remains unproven for ankylosing spondylitis. Since carotid intima-media thickness is an indicator of early atherosclerosis, and the paraoxonase (PON1) enzyme has antioxidant activity to prevent LDL oxidation, we aimed to identify: 1) the relationship between carotid intima-media thickness (CIMT) and serum paraoxonase (PON1) activity in ankylosing spondylitis (AS) patients; and 2) the possible differences in CIMT in AS patients versus age-matched, healthy controls.

Methods: Forty-five AS patients (36.8±9.8 years, 36 males, 9 females) and 30 controls (35.9±10.2 years, 23 males, 7 females) were recruited consecutively. Serum PON1 activity and CIMT were measured. The Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Radiologic Index (BASRI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) were used to identify relationships between these clinical indices and levels of CIMT and PON1.

Results: Mean CIMT was significantly increased in AS patients relative to controls (0.49±0.06 mm vs. 0.59±0.07 mm; $p < 0.0001$). Conversely, serum PON1 activity was decreased (199.1±60.3 U/L vs. 96.7±29 U/L; $p < 0.0001$). PON1 activity was negatively correlated with CIMT ($r = -0.557$, $p = 0.0001$). Disease duration was positively correlated with CIMT ($r = 0.542$, $p = 0.0001$) and negatively correlated with PON1 ($r = -0.649$, $p = 0.0001$). On multivariate analysis, disease duration and serum PON1 activity were found to be independent predictors of CIMT ($R^2 = 0.687$, $p = 0.0001$).

Conclusions: In conclusion, significantly increased CIMT and decreased PON1 activity suggest a relationship between atherosclerosis and AS: a relationship that is strongly correlated with disease duration.

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In recent years, studies of various rheumatological disorders have demonstrated that these patients have an increased risk of developing atherosclerosis [1]. Such a relationship has been clearly described for rheumatoid arthritis and systemic lupus erythematosus [2].

Ankylosing spondylitis (AS) is an inflammatory disease characterized by arthritis and enthesitis in the spine and peripheral joints [3]. Patients with AS are known to have an overall mortality rate roughly 1.6–1.9 times that of the general population, and excess mortality from circulatory or cardiovascular diseases has been estimated at 20–40% [4,5].

Currently, atherosclerosis can be demonstrated by non-invasive imaging techniques. For this purpose, common carotid artery intima-media thickness (CIMT) currently has been used as a clinically useful, low-cost and non-invasive way of identifying atherosclerosis [6]. In two studies, a relationship between chronic inflammation and atherosclerosis has been identified [7,8]. In these studies, endothelial functions have been shown to be disrupted in the common carotid artery (CCA) and coronary arteries.

PON1 is a Ca^{2+} -dependent serum esterase, composed of 354 amino acids (43 kDa), that is synthesized in the liver [9]. It is a high density lipoprotein (HDL)-associated enzyme with three actions: paraoxonase, arylesterase and dyazoxonase [10]. Paraoxonase-1 (PON1) is principally complexed with HDL in human serum, and has been implicated in the protection of LDL and HDL from the oxidation that is induced by copper ions, as well as by other free radical generators. This protection is likely associated with the PON1 hydrolyzing activity of some activated phospholipids and lipid peroxide products [11,12]. Thus, PON1 prevents the acceleration of atherosclerosis and assumes anti-atherogenic properties [13].

To our knowledge, PON1 activity has not been studied in AS patients. In this study, we objectively assessed PON1 activity in AS patients by measuring CIMT.

Materials and Methods

Subjects and study design

Forty-five AS patients (36.8 ± 9.8 years, 36 males, 9 females) were enrolled into the study from among patients who presented to the Physical Medicine and Rehabilitation Unit at Harran University School of Medicine between February and July 2010. All patients fulfilled the modified 1984 New York criteria for AS [14]. Thirty healthy controls (35.9 ± 10.2 years, 23 males, 7 females) matched for age, sex and body mass index (BMI) were recruited from the staff of the same hospital. Prior to subject recruitment, the research protocol was approved by

the Ethics Committee of Harran University School of Medicine, and written informed consent was obtained from all subjects. Patients and controls were excluded if they reported any of the following, or if any of the following were detected in their medical records, or on physical examination, imaging or laboratory work-up: myocardial infarction, active infectious disease, auto-immune disorders including SLE, Sjogren disease, RA, spondyloarthropathies other than AS, crystal arthropathies and vasculitis, major depression, liver disease, neoplastic disease, diabetes mellitus, hypertension, renal failure, family history of premature coronary heart disease (< 55 years in males, < 65 years in females), and iron deficiency anemia. Smokers and those who regularly consumed alcohol also were excluded, as were individuals taking any drug with anti-oxidant properties, such as beta-blocking agents (carvedilol, nebivolol), angiotensin-converting-enzyme inhibitors (captopril, zofenopril), statins, diuretics, or vitamins. In the study, all AS patients (26/26) were on NSAIDs, 25/26 were on sulphasalazine and 5/26 were using methotrexate. None of the patients was using TNF- α blockers.

Laboratory evaluation

In the morning after an overnight fast, venous blood was sampled for the measurement of serum concentrations of glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, C-reactive protein, and paraoxonase activity. The samples were centrifuged at 3,000 rpm for 10 minutes. Serum samples reserved for specific parameters were stored at -70°C until the day of analysis.

Measurement of paraoxonase activity

Measurements of paraoxonase activity were performed in the absence of basal activity. The rate of paraoxon hydrolysis (diethyl-*p*-nitrophenylphosphate) was measured by monitoring the increase of absorbency at 412 nm at 37°C . The amount of generated *p*-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was $17000 \text{ M}^{-1} \text{ cm}^{-1}$ [15]. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol: $1310 \text{ M}^{-1} \text{ cm}^{-1}$. One unit of arylesterase activity was defined as $1 \mu\text{mol}$ phenol generated min^{-1} under the above conditions and expressed as U/L serum [16].

TABLE 1. Comparison of laboratory findings, demographic and clinical characteristics in patients with ankylosing spondylitis and controls

	AS patients (n=45)	Controls (n=30)	p-value
Age (years)	36.8±9.8	35.9±10.2	0.706
Male/female (number)	36/9	23/7	
Disease duration (years)	12.8±6.3		
Height (cm)	167.1±6.9	169.1±6.5	0.225
Weight (kg)	70.7±12.8	71.2±10.5	0.882
BMI (kg/m ²)	25.3±4.1	24.5±3.7	0.364
BASFI	4.8±2.1		
BASDAI	4.16±1.8		
BASMI	4.76±2.0		
BASRI	8.5±3.7		
ESR (mm)	26.8±10.6	8.5±3.5	0.0001
Plasma glucose (mmol/l)	4.38 ± 0.61	4.31 ± 0.55	0.625
Total cholesterol (mmol/l)	4.21 ± 0.99	3.99 ± 0.86	0.315
LDL cholesterol (mmol/l)	2.65 ± 0.76	2.55 ± 0.59	0.522
HDL cholesterol (mmol/l)	1.05 ± 0.19	1.07 ± 0.13	0.534
Triglyceride (mmol/l)	1.50 ± 0.64	1.38 ± 0.50	0.397

Sonographic study

All of the sonographic examinations were performed by the same examiner, who was unaware of the subjects' clinical status throughout the study. Each subject was studied in the morning hours (8:00 a.m. to 10:00 a.m.) after having abstained from alcohol, caffeine, tobacco and food for 8 hours before the examination. None of the participants was using vasoactive drugs. Studies were performed in a quiet, temperature-controlled room (20–25°C). Images were obtained by high resolution Doppler ultrasound (Logiq 7 Pro; General Electric, Milwaukee, WI, USA) with a 12 MHz linear-array transducer. All sonographic examinations were evaluated by the same investigator to avoid inter-observer variations.

Bilateral assessment of wall thickness was made in the common carotid artery (CCA). Intima-media thickness (IMT) was measured as the distance from the leading edge of the first echogenic line to that of the second echogenic line. The first line represents the lumen–intima interface, and the second line the collagen-containing upper layer of the tunica adventitia. IMT measurement of both the right and left CCA was performed at three points along the far wall in each CCA from 2 cm proximal to the bifurcation of the CCA. The three locations were then averaged to produce the mean IMT for each side. The average of the two sides was considered the patient's overall mean CIMT.

TABLE 2. Common carotid artery IMTs and PON1 activity levels in ankylosing spondylitis patients and controls

	AS patients (n=45)	Controls (n=30)	p-value
Right CCA IMT (mm)	0.60±0.08	0.50±0.06	<i>p</i> <0.0001
Left CCA IMT (mm)	0.58±0.05	0.49±0.07	<i>p</i> <0.0001
mean CCA IMT (mm)	0.59±0.07	0.49±0.06	<i>p</i> <0.0001
PON 1 activity (U/L)	96.7±29.7	199.1±60.3	<i>p</i> <0.0001
Arylesterase (U/L)	90.0±17.5	119.0±16.8	<i>p</i> <0.0001

Other measurements

Height, weight, waist circumference and waist/hip ratio (WHR) were recorded for each subject. BMI was calculated according to Quetelet's index, as the ratio of weight to height squared (kg/m²). Blood pressure was measured with a mercury column sphygmomanometer. Spinal mobility was assessed using the Bath Ankylosing Spondylitis Metrology Index (BASMI) [17]. Clinical indices of disease activity (Bath Ankylosing Spondylitis Disease Activity Index; BASDAI) [18], radiographs of sacroiliac joints, lumbar spine, and cervical spine were assessed by using the bath ankylosing spondylitis radiologic index (BASRI) [19] with a possible range of 0–12, and functional status (Bath Ankylosing Spondylitis Functional Index; BASFI) [20] also were generated.

Statistical analysis

All data analysis was conducted using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA), with group parameters expressed as means ± standard deviations. Between-group comparisons were conducted by independent samples Student's *t*-tests for continuous variables. Bivariate correlations were performed using Pearson's correlation analysis, for which an *a priori* designation system was adopted: *R* < 0.40, weakly correlated; *R* = 0.40–0.69, moderately correlated; and *R* > 0.70, strongly correlated. Multivariate analyses were performed with linear regression models. Differences at *p* < 0.05 were interpreted as statistically significant. All inferential tests were two tailed.

Results

The demographical data of patients and controls are shown in Table 1. No differences in TG, LDL, HDL or total cholesterol were apparent between AS patients and controls. Serum PON1 activity was significantly decreased (*p* < 0.0001) and mean carotid intima-media thickness considerably increased in AS patients versus controls (Table 2). Disease duration was

FIGURE 1. Correlations between mean CIMT and a) PON1 and b) arylesterase activity

positively correlated with BASRI ($r = 0.506$, $p = 0.0001$) and BASMI ($r = 0.322$, $p = 0.031$). BASFI and BASDAI indices were not significantly correlated with disease duration. Disease duration was positively correlated with mean CIMT ($r = 0.542$, $p = 0.0001$) and negatively correlated with PON1 ($r = -0.649$, $p = 0.0001$). PON1 activity and arylesterase levels were negatively correlated with mean CIMT ($r = -0.557$, $p = 0.0001$ and $r = -0.34$, $p = 0.022$; respectively). Erythrocyte sedimentation levels were negatively correlated with PON1 and arylesterase levels but these correlations were statistically insignificant ($r = -0.54$, $p = 0.7$ and $r = -0.200$ and $p = 0.188$, respectively). On the other hand, ESR levels were positively but insignificantly correlated with mean CIMT ($r = 0.270$, $p = 0.064$). In a regression model, where mean CIMT was the independent variable and PON1, age, disease duration, BASMI, and BASRI dependent variables, disease duration and PON were found to be independent predictors of CIMT ($\beta = 0.233$, $p = 0.018$; $\beta = -0.046$, $p = 0.001$, respectively). Adjusted R^2 was 0.687 and p was 0.0001 for this model.

Discussion

In rheumatologic disorders, mortality rates are significantly higher than in the general population. This difference can be attributed primarily to cardiovascular diseases.²¹⁻²³ Several re-

cent studies have demonstrated the role of chronic inflammation in endothelial damage and the development of atherosclerosis [24]. Atherosclerosis affects whole arterial layers; its most prominent effect being in the intima-media layer. Ultrasonographic measurement of carotid intima-media thickness (CIMT) is a reliable indicator of early atherosclerosis [25,26].

The relationship between atherosclerosis and chronic inflammation has been a growing area of interest for certain rheumatologic diseases, including SLE and RA. To date, any relationship between AS and atherosclerosis is merely speculative. In our study, several important results were generated. First, CIMT measurements were significantly higher in those with AS versus controls. As mentioned above, increased CIMT is a reliable indicator of early atherosclerosis. Our results must be considered with those of prior studies that produced conflicting results. Mathiue *et al.*, [27] for example, revealed a significant increase in CIMT among AS patients; but Choe *et al.* failed to identify any difference [28]. These disparate results may have arisen from differences in measurement techniques, different study populations, and/or, most importantly, differences in disease duration. In our study, the mean duration of AS was 12.8 years: a reasonable duration of time during which atherosclerosis could begin and progress.

Another important result in our study relates to disease duration, which we found to be positively correlated with

CIMT. This makes sense, because the development of atherosclerosis is time-dependent and progressive. Again, patients were primarily selected whose disease had been active for several years, since newly-diagnosed AS patients would not be expected to have AS-induced atherosclerosis.

The disease duration was also found to be significantly correlated with both the BASMI and BASRI ($r = 0.506$, $p = 0.0001$ and $r = 0.322$, $p = 0.031$), but not the BASFI and BASDAI. This finding is intuitive, since the BASMI index is calculated by metric measurement of joint movements and the BASRI is generated from radiological findings, both of which require time for development. Conversely, the BASDAI and BASFI indices demonstrate current disease activity and functionality. In other words, since it takes years to develop permanent changes in AS patients (as measured metrically or shown radiologically), that is why BASMI and BASRI indices correlated with disease duration. Instant or daily disease activity, that is, disease activity which develops over a relatively short period of time, might not be expected to correlate with disease duration, since disease activity can vary day to day or week to week. So, that is possibly why BASDAI and BASFI indices did not correlate with disease duration.

A third important discovery relates to oxidative stability, which is defined as the balance between the formation and elimination of free radicals. Any increase in the rate of free radical formation or decrease in their elimination can disrupt this balance, resulting in what has been called oxidative stress [29]. Oxidative stress alters normal endothelial functions, inducing proinflammatory, prothrombotic, proliferative and vasoconstrictor mechanisms that support atherogenic processes. A number of investigators have implicated oxidative stress and/or inadequate antioxidant defences in the pathogenesis of, or as a risk factor for, cardiovascular disease. Ischemic heart disease is an ideal example of a clinical situation in which there is increased production of oxygen free radicals. Oxidative stress has been regarded one contributor to the progression of atherosclerosis, since oxidated low density lipoprotein plays a key role in atherosclerosis development. Under physiologic conditions, free radical generation is controlled by a large number of free-radical antagonist (antioxidant) systems that act to prevent and/or inhibit the harmful effects of free radicals. As mentioned previously, the PON1 enzyme exhibits significant anti-oxidant properties and plays an important role in preventing LDL oxidation. Taken together, these observations mean that the PON1 enzyme acts primarily as an antioxidant, especially preventing LDL oxidation, and hence seems to play a preventative role in atherogenesis [30,31].

A fourth point that should be made is that PON1 activity was found to be significantly decreased in AS patients. This finding is consistent with the observed elevated CIMT levels, in terms of early atherosclerosis. As mentioned above, LDL oxidation in arterial walls is a major step in atherogenesis [32]. PON1 has antioxidant properties, and it is well known from epidemiological data that HDL exerts cardio-protective effects through its anti-oxidant activity, which is largely maintained by PON [9]. PON hydrolyzes organophosphates and lipid peroxide (LPO) products and neutralizes the harmful effects of LPO in LDL. Thus, PON1 is presumed to prevent the acceleration of atherosclerosis [13].

Serum PON activity is generally considered to vary in response to the consumption of PON1 for prevention of oxidation [33]. The significantly decreased PON1 activity observed in this study may reflect the increased consumption of antioxidant activity resulting from chronic inflammation and oxidant stress. Contrary to our results, Erdem *et al.* reported no difference in PON or arylesterase levels between AS patients and controls, but this may have been the result of different study techniques and/or shorter disease duration [34]. On the other hand, PON1 activity was found to be negatively correlated with disease duration, suggesting that PON1 is consumed as the disease progresses and chronic inflammation continues, which possibly is indicative of increased LDL oxidation and subsequent atherosclerosis. Moreover, PON levels correlated with the BASRI, but not with the BASDAI score, again indicating its relationship with disease duration.

Regression analysis bore all of this conjecture out, as PON1 and disease duration were the two independent determinants of CIMT among AS patients. The BASMI and BASRI indices were not found to be determinants of CIMT, when entered into the regression model along with PON1 and disease duration. This is not surprising, given that disease duration is the main determinant of BASRI and BASMI. In other words, because of co-linearity, they were not the primary determinants of CIMT. Whether directly or indirectly, PON1 activity seems to be an important predictor of CIMT. PON1, in turn, might be affected by other factors, so the correlation could be the consequence of several pathways that indirectly affect CIMT. At this stage of our understanding, whether the observed increase in PON1 levels was a consequence or result is not important. What matters is that the PON1 levels reflected changes in CIMT measurements.

To summarize, in our study of 45 AS patients and 30 healthy controls, CIMT was found to be significantly higher in AS patients. Disease duration and PON1 activity both appeared to affect CIMT, whether directly or indirectly. Third,

though the BASRI and BASMI indices correlated with disease duration, they failed to do so with CIMT. Based upon these results, we feel that AS patients are, indeed, predisposed to atherosclerosis, and that PON1 might be used as a biochemical marker for this process; however, further studies are needed.

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