

Relationship among plasma secretory phospholipase A₂, oxidized low density lipoprotein & paraoxonase activities in hypertensive subjects treated with angiotensin converting enzyme inhibitors

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Background & objectives: Secretory phospholipase A₂ (sPLA₂) and oxidized low density lipoprotein (oxLDL) are considered as oxidative and inflammatory markers. The effects of oxLDL have been shown to be inhibited by paraoxonase (PON1). This study was undertaken to investigate the relationship between oxidative and inflammatory markers in hypertensive patients with or without antihypertensive drug treatment.

Methods: Newly diagnosed hypertensive patients (n=35) and hypertensive patients who had been taking angiotensin converting enzyme (ACE) inhibitors as antihypertensive therapy (10 or 20 mg/day for 9 ± 2 wk; n=35) and age-matched normotensive subjects (controls; n=20) were included in this study. Plasma sPLA₂, oxLDL and PON1 activities were determined.

Results: Hypertensives had higher plasma oxLDL and sPLA₂ levels ($P<0.01$) and lower PON1 levels than the controls ($P<0.01$). Treated hypertensives had lower plasma sPLA₂ and oxLDL levels and higher PON1 activities than untreated hypertensives ($P<0.01$). sPLA₂ was positively correlated with oxLDL ($r=0.433$, $P<0.01$) and negatively correlated with plasma PON1 ($r= - 0.540$, $P<0.01$) in untreated hypertensives. In controls and treated hypertensives, plasma PON1 was positively correlated with oxLDL ($r= 0.455$, $r=0.429$, $P<0.01$, respectively) and sPLA₂ ($r= 0.450$, $r=0.506$, $P<0.01$, respectively).

Interpretation & conclusions: Reduction in PON1 activity and elevation in both sPLA₂ activities and oxLDL levels might be involved in elevated oxidative stress and inflammation. ACE inhibitor treatment may help reduce inflammation and oxidative stress in hypertensives.

Key words Hypertension - inflammation markers - oxidized low density lipoprotein - paraoxonase - secretory phospholipase A₂

The pathophysiology of essential hypertension and its complications has been a focus of research and clinical interest. Recent attention has been directed towards endothelial dysfunction¹. Endothelial dysfunction is involved in lesion formation by the

promotion of both the early and late mechanisms of atherosclerosis including upregulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced low density lipoprotein (LDL) oxidation,

platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration².

The link between oxidative stress and endothelial dysfunction was originally derived from the oxidative modification hypothesis as a key step in the generation of macrophage-derived foam cells and the initiation of atherosclerosis³. Inflammation is an integral feature of atherosclerosis, in which inflammatory processes contribute to the initiation, progression and rupture of lipid-rich atherosclerosis plaques. Secretory phospholipase A₂ (sPLA₂) and oxidised (oxLDL) are involved in inflammation and oxidative stress, and are accepted as oxidative and inflammatory markers⁴.

Generated reactive oxygen species (ROS) induces the oxidative modification of LDL, which leads to the formation of oxLDL and deleterious lipids such as lysophosphatidylcholine (LPC). These atherogenic lipids have a potent activity to induce endothelial dysfunction and lesion formation⁵. Secretory phospholipase A₂ (sPLA₂) enhances LDL oxidation, and promotes the formation of bioactive phospholipids via the release of polyunsaturated free fatty acids, resulting in an enhanced ability to stimulate monocyte-endothelium interaction⁶. On the other hand, the effects of oxLDL in the development of atherosclerotic process have been shown to be inhibited by paraoxonase (PON1), an HDL-associated esterase⁷. PON1 deficiency is associated with increased macrophage-oxidative stress, where an effect on LDL oxidation was observed⁸. PON1 plays a role in preventing lipid peroxidation not only of LDL but also of HDL⁹. Only small increases in HDL concentrations have been shown to greatly reduce atherogenicity and this effect has been confirmed to be related to increased PON1 activities⁷. However, the mechanism by which PON1 protects against oxidative damage and consequently development of atherosclerosis is not entirely clear.

Many studies have been undertaken to investigate relationship between blood pressure/hypotensive drug treatment and PON1/lipid peroxidation status^{10,11}. The relationship between sPLA₂ and oxLDL levels has not been much investigated in patients with hypertension. We therefore undertook this study to evaluate the relationship between sPLA₂ activities and oxLDL levels and PON1 activities in hypertensive patients with or without treatment.

Material & Methods

The study was conducted at the Department of Biochemistry in Cerrahpasa Medical Faculty, Istanbul

University, Turkey, and Department of Biochemistry, Taksim Education and Research Hospital, Turkey. During 2004 and 2006, 35 newly diagnosed drug naïve hypertensive patients (mean age \pm SD: 48.1 \pm 7.7 yr); and 35 hypertensive patients who had been taking 10 or 20 mg once daily angiotensin converting enzyme (ACE) inhibitors as an antihypertensive treatment for 9 \pm 2 wk (47.7 \pm 6.7 yr) were included in the study. Subjects who were admitted by Department of internal medicine were randomise selected. Blood samples were taken for patients during routine examination. Age and sex matched normotensive subjects (controls, N=20, mean age: 42.8 \pm 9.5 yr) were voluntarily selected from healthy laboratory workers. All subjects gave informed written assent and verbal informed consent prior to the study.

Hypertension was diagnosed when the systolic pressure was 140 mmHg or greater, and the diastolic pressure was 90 mmHg or greater. Measurements were obtained with the subjects in the sitting position after resting for 20-30 min¹². Measurements were performed on three different times. The average of three measurements was taken as the mean systolic and diastolic pressures.

BMI was measured as weight/ height². The waist-to-hip ratio (WHR) was calculated¹³. Exclusion criteria included pregnancy, cardiovascular diseases, renal, metabolic or hormonal diseases, hyperinsulinaemia (fasting insulin above 15 μ U/ml), smoking, alcohol abuse, or receiving lipid-lowering therapy, vitamins or antioxidants. All subjects were free of concomitant vascular disease, malignancy or connective tissue diseases.

After 12 h overnight fasting, 5 ml venous blood samples from all participants were drawn into Li-heparin containing tubes, and were centrifuged at 1500 g for 10 min in a refrigerated centrifuge to obtain plasma. Blood samples were obtained from hypertensive subjects before anti-hypertensive therapy. The samples were kept in -80°C until analysis of plasma sPLA₂, oxLDL and PON1 analyses were performed within 2 wk. The other biochemical measurements were performed on the sampling day.

Plasma sPLA₂ activity was measured using a specific substrate sPLA₂ that is converted into a sulfhydryl (sPLA₂ enzyme assay, R&D Systems Inc., USA). The intra- and inter-assay coefficients of variation were 4.6 and 4.5 per cent, respectively.

Plasma oxLDL levels were directly measured by a sandwich ELISA assay (Mercadia Oxidized LDL ELISA, Uppsala, Sweden). The intra- and inter-assay coefficients of variation were 5.1 and 9.3 per cent respectively. Data were expressed as units per liters (U/l).

Plasma PON1 activity (U/l) was measured using synthetic paraoxon (diethyl p-nitro phenyl phosphate) as substrate¹⁴. The intra- and inter-assay coefficients of variation were 5.6 and 8.5 per cent, respectively.

Levels of plasma glucose, total cholesterol, HDL cholesterol, triglyceride and creatinine were determined using commercial kits (Roche Diagnostics, GmbH, and Mannheim, Germany). LDL cholesterol was calculated using the Friedewald's formula¹⁵ if the triglycerides were less than 4.5 mmol/l. Plasma insulin levels were determined by solid-phase-two-site chemiluminescent immunometric assay (Immulite, Euro/DPC Ltd., USA). All chemicals were obtained from Sigma, USA.

Statistical analysis: Comparisons were made using the unpaired t test and ANOVA followed by Bonferroni post-hoc test for data between groups. Correlations between changes in variables were tested using Pearson's correlation. $P < 0.05$ was considered statistically significant.

Results

Age, gender distribution, BMI and WHR did not differ among the groups. There was no difference in blood pressure levels between controls and treated hypertensives. Systolic and diastolic blood pressure of newly diagnosed hypertensives was significantly higher than controls and treated hypertensives ($P < 0.01$). Plasma lipids, glucose, insulin and creatinine levels were not different among the groups (Table I).

Both groups of hypertensives (with and without treatment) had significantly higher sPLA₂ activities and oxLDL levels and significantly lower PON1 activities than the controls ($P < 0.01$). Treated hypertensives had significantly lower plasma sPLA₂ activities and oxLDL levels and significantly higher PON1 activities compared to untreated hypertensives ($P < 0.01$) (Table II).

PON1 levels in controls and treated hypertensives' groups was positively correlated with oxLDL ($r = 0.455$, $r = 0.429$, $P < 0.01$, respectively) and sPLA₂ ($r = 0.450$, $r = 0.506$, $P < 0.01$, respectively). In untreated hypertensive group, sPLA₂ levels was positively

Table I. General and plasma biochemical characteristics of controls, hypertensives and treated hypertensives

	Controls (n=20)	Hypertensives (n=35)	Treated- hypertensives (n=35)
Age (yr)	42.8 ± 9.5	48.1 ± 7.7	55.7 ± 9.7
BMI (kg/m ²)	27.4 ± 2.5	28.5 ± 1.9	28.8 ± 2.3
Sex (female/male)	10/10	19/16	17/18
Waist-hip-ratio	0.91 ± 0.06	0.90 ± 0.07	0.89 ± 0.11
SBP (mmHg)	100.5 ± 10.5	163.5 ± 18.5*	104.5 ± 12.5 ⁺
DBP (mmHg)	74.4 ± 5.5	98.5 ± 6.5*	77.6 ± 5.5 ⁺
Glucose (mmol/l)	5.24 ± 1.27	5.31 ± 0.90	5.37 ± 0.87
Total cholesterol (mmol/l)	4.92 ± 0.54	4.94 ± 0.64	4.93 ± 0.82
LDL- cholesterol (mmol/l)	3.39 ± 0.81	3.36 ± 0.88	3.41 ± 0.69
HDL- cholesterol (mmol/l)	1.24 ± 0.20	1.25 ± 0.22	1.23 ± 0.35
Triglycerides (mmol/l)	1.46 ± 0.29	1.47 ± 0.34	1.47 ± 0.45
Creatinine (μmol/l)	70 ± 13	69 ± 12	71 ± 18
Plasma insulin (μU/ml)	10.1 ± 3.2	11.5 ± 3.9	10.5 ± 3.5

Values are means ± SD

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure

* $P < 0.01$ vs controls (ANOVA and Tukey post-hoc test)

⁺ $P < 0.01$ vs hypertensives (ANOVA and Tukey post-hoc test)

Table II. Plasma oxidized low density lipoprotein (oxLDL), paraoxonase-1 (PON1), and secretory phospholipase A₂ (sPLA₂) in controls, hypertensives and treated-hypertensives

	Controls (n=20)	Hypertensives (n=35)	Treated- hypertensives (n=35)
sPLA ₂ (U/ml)	0.44 ± 0.21	0.79 ± 0.40*	0.62 ± 0.31* ⁺
OxLDL (U/l)	40.8 ± 7.5	67.5 ± 6.7*	50.9 ± 9.5* ⁺
PON1 (U/l)	62.3 ± 11.5	35.6 ± 10.5*	50.5 ± 9.5* ⁺

Values are means ± SD

* $P < 0.01$ vs controls (ANOVA and Tukey post test)

⁺ $P < 0.01$ vs hypertensives (ANOVA and Tukey post test)

correlated with oxLDL levels ($r = 0.433$, $P < 0.01$) and negatively correlated with PON1 levels ($r = -0.540$, $P < 0.01$).

Discussion

Hypertension and endothelial dysfunction are associated diseases although the underlying mechanisms are not completely known. It is possible that each of the two conditions predisposes to and/or aggravates the other. The endothelial injury, activation, and dysfunction are caused by oxLDL in the pathogenesis of atherosclerosis¹⁶. The exact cause

and effect relationship between oxLDL components and atherogenic events remains largely speculative. Raised serum levels of oxLDL *in vitro* have been found in hypertensive patients¹⁷.

In our study, we confirmed that oxidative modification of LDL levels and elevation in hypertensive stage were reversed by ACE inhibitors. We also found that the PON1 activities of the hypertensive patients were lower than the normotensives. PON1 prevents LDL oxidation and also renders HDL resistant to oxidation, thereby maintaining the capacity of HDL to induce reverse cholesterol transport¹⁸. Increase in HDL-cholesterol is suggested to be associated with decreased risk of coronary artery diseases¹⁹. Although there was no significant difference in plasma HDL-cholesterol levels between normotensives and hypertensives, it might be possible that the antioxidant effect of HDL was decreased due to the elevation in oxidative stress levels. While plasma oxLDL activities were found to be negatively correlated with PON1 in hypertensives, oxLDL activities were positively correlated with PON1 in the normotensives. This suggests that a well-balanced antioxidant/oxidant status was present in healthy conditions.

In our study sPLA₂ levels were positively correlated with oxLDL and negatively correlated with PON1 in hypertensive group. This finding may be attributed to the loss of PON1 activity from sPLA₂-modified HDL. It has been reported that sPLA₂ liberates polyunsaturated fatty acids from not only LDL but also HDL and sPLA₂-modified HDL loses its protective properties against LDL oxidation^{20,21}.

In our patients on antihypertensive drug PON1 activities were higher and oxLDL and sPLA₂ levels lower than the drug naive hypertensives. It has been proposed that an interaction between oxLDL and renin-angiotensin system (RAS) activation is a major determinant of atherogenesis²². OxLDL accumulation in the blood vessels enhances the expression and activation of RAS components; on the other hand, activation of RAS stimulates LDL oxidation in the blood vessels²³. Oxidative stress was decreased and the oxidant-antioxidant balance improved by using ACE inhibitors in hypertensives as also reported earlier²⁴.

The elevation of oxidative stress may be independent of the changes of blood pressure levels, as well as BMI or age. It has been found that PLA₂ was not related with BMI and blood pressure^{25,26},

and Frostegard *et al*¹⁶ indicated that oxLDL was elevated in hypertensives and was independent of the differences in plasma cholesterol or BMI. Contrary to our findings, Uzun *et al*²⁷ reported that the oxLDL and PON1 levels were dependent on the difference in the blood pressure levels. Saruhan *et al*²⁸ suggested that serum PON1 activity and HDL cholesterol levels remained unchanged with age and gender in Turkish population. Additionally, it has been reported that the gene frequency for the PON1, 192/55 polymorphisms in healthy Turkish people was not significantly different from that of coronary artery disease patients²⁹.

Our study had several limitations. Plasma lipid levels were in similar range in the studied groups, therefore, the relationship between plasma lipids and studied markers were not clearly demonstrated. As the number of subjects was limited, the findings need further confirmation.

In conclusion, our data suggested that an imbalance between oxidant and antioxidant status in hypertension was related with reduction in PON1 activity and elevation in both sPLA₂ activity and oxLDL levels. There were a relationship between PON1 and sPLA₂ activities and interactions between these markers played different roles in pathogenesis of hypertension. Pharmacological approaches that target the renin-angiotensin system may reduce oxidative stress in hypertension. Further studies are needed to asses the increased cardiovascular risk related with sPLA₂, PON1 and oxLDL levels.

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