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ORIGINAL ARTICLE

Is there a relationship between serum ox-LDL, oxidative stress, and PON1 in knee osteoarthritis?

Cemil Ertürk[1] · Mehmet Akif Altay[2] · Ali Bilge[3] · Hakim Çelik[4]

Abstract Previous in vitro studies have shown that oxidized low-density lipoprotein (ox-LDL) plays a role in the pathogenesis of osteoarthritis (OA). Paraoxonase-1 (PON1) protects both low-density lipoproteins (LDLs) and high-density lipoprotein (HDLs) against oxidative damage from circulating cells. In addition, PON1 is inactivated by ox-LDL and preserved by antioxidants. However, the relationship between serum ox-LDL, oxidative stress, and PON1 in knee OA remains unclear. Therefore, we investigated ox-LDL association with oxidative stress and PON1 in knee OA, and evaluated their relationships using radiological and clinical parameters. This study included 203 patients and 194 controls. The severity of OA was classified based on the Kellgren–Lawrence scoring system. In addition, each patient was clinically evaluated using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) score. Plasma concentrations of ox-LDL, oxidative stress markers, and PON1 were measured. Serum ox-LDL and oxidant parameters were significantly higher in patients compared to controls ($p < 0.001$ for all), whereas PON1 was significantly lower ($p < 0.001$). ox-

LDL was inversely correlated with PON1, whereas it was positively correlated with radiographic severity, WOMAC score, and oxidant parameters. We found an association between the levels of various serum markers of oxidative injury, especially ox-LDL, and increasing severity of knee OA, as well as indirect evidence for their regulation by PON1. oxLDL seems to play a critical role in OA, both in the beginning, and during progression of, the disease. Therefore, serum oxLDL levels may be a helpful biomarker to evaluate the severity of knee OA.

Keywords Oxidative stress · Ox-LDL · PON1 · Severity of knee OA

Introduction

Previous in vitro studies have reported that oxidized low-density lipoprotein (ox-LDL) plays a role in the pathogenesis of articular cartilage degeneration [1–3]. ox-LDL is a lipid peroxidation product induced by oxidative stress that contributes to inflammation through specific gene expression [4, 5]. It has been shown that ox-LDL plays a critical role in the development of atherosclerosis [6, 7]. It has also been reported that ox-LDL may be involved in the pathogenesis of osteoarthritis (OA) [8, 9]. Indeed, this molecule stimulates reactive oxygen species (ROS) production and promotes cartilage degradation due to collagen breakdown [1, 4, 5, 10].

Previously, we examined Paraoxonase-1 (PON1) and oxidative stress in knee OA [11]. PON-1 is a hydrolytic enzyme associated with high-density lipoprotein (HDL). It protects both low-density lipoproteins (LDLs) and HDLs against oxidative damage from circulating cells [12–14]. In addition, PON1 is inactivated by ox-LDL and preserved by antioxidants [15, 16]. To the best of our knowledge, there is no report

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on the relationship between serum ox-LDL, oxidative stress, and PON1 in knee OA in the English literature.

Therefore, we sought to address the following questions: (1) Is there a relationship between ox-LDL and oxidative stress markers in knee OA?; (2) Is there a relationship between ox-LDL and PON1 in knee OA?; and (3) Is there a relationship between ox-LDL, radiographic severity, and clinical parameters?

Materials and methods

Study population

This cross-sectional study included 203 patients with OA (161 females and 42 males) and 194 healthy controls (147 females and 47 males). The diagnosis of OA was confirmed based on American College of Rheumatology criteria [17]. Exclusion criteria were consistent with those previously described [11]. Controls were selected on the basis of having a similar distribution to the cases in terms of age, body mass index (BMI), and gender. For inclusion in the control group, subjects had to have no known knee OA symptoms. This study was approved by the local Ethics Committee and written informed consent was obtained from all patients.

Radiologic and clinical assessment

Conventional knee radiographs were taken for radiologic assessment as previously described [18]. Radiographic grading of the knee OA was evaluated utilizing the Kellgren-Lawrence (KL) scoring system [19].The Western Ontario and McMaster University Osteoarthritis Index (WOMAC) was used clinical evaluation [20].

Biochemical analysis

The serum was prepared by centrifugation at 3000 rpm for 10 min. All samples were stored at −80 °C until these were analysed. The levels of triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDL-C), and LDL-cholesterol (LDL-C) were measured using enzymatic methods (Abbott, Wiesbaden-Delkenheim, Germany) with an autoanalyser (Aeroset®; Abbott). ox-LDL concentrations were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) method (Immundiagnostik, Cat no: 201–12-1543) [21]. ELISAs were carried out according to the manufacturer's instructions. Serum PON1 and plasma concentration were measured together with other enzymes of oxidative status [lipid hydroperoxide (LOOH), total oxidative status (TOS), and the oxidative stress index (OSI)] and antioxidative status [(free sulfhydryl groups, −SH = total thiol), and total

antioxidative capacity (TAC)].The methods were determined as previously described [11, 22, 23].

Statistical methods

All statistical analyses were conducted using the SPSS 16 computer program. Chi-square test and Student's t-test and one-way ANOVA were used for group comparisons. For correlation analysis, a Pearson's correlation test was used. Standardized β-values in multiple linear regressions were described. Values of $P < 0.05$ were significant.

Results

The demographic characteristics of the study participants are shown in Table 1. There was no statistical difference between patients and control subjects in age, BMI, female/male ratio and in terms of fasting blood triglyceride, total cholesterol, or LDL and HDL levels ($p > 0.05$). The ox-LDL and oxidative stress were significantly higher in OA, whereas PON1 was significantly lower in OA ($p < 0.001$). Furthermore, there was no significant difference between the two groups ($p > 0.05$) according to antioxidative capacity.

We divided our patients into four subgroups according to the KL scoring system: Grade 1 ($n = 50$), grade 2 ($n = 54$),

Table 1 Demographic characteristics of the knee osteoarthritis and control groups

	Patients* ($n = 203$)	Controls* ($n = 194$)	p-value
Age (years)	58.1 ± 9.6	56.3 ± 10.1	>0.05
Gender (M:F)	42/161	47/147	>0.05
BMI (kg/m^2)	30.1 ± 3.3	30.5 ± 3.9	>0.05
TG (mmol/L)	176.1 ± 75.9	170.7 ± 73.6	>0.05
TC (mmol/L)	205.0 ± 37.0	206.9 ± 37.8	>0.05
HDL (mmol/L)	44.8 ± 13.7	44.9 ± 13.5	>0.05
LDL (mmol/L)	122.7 ± 34.6	124.6 ± 34.8	>0.05
Ox-LDL (mmol/L)	13.8 ± 4.3	12.3 ± 4.0	<0.001
Paraoxanase activity (U/L)	77.2 ± 24.7	99.5 ± 21.4	<0.001
LOOH (μmol/L)	51.4 ± 21.6	30.0 ± 16.5	<0.001
TOS (μmol H$_2$O$_2$ equiv./L)	81.4 ± 32.7	46.0 ± 23.1	<0.001
OSI (Arbitrary unit)	8.4 ± 3.8	4.7 ± 2.5	<0.001
Total –SH (mmol/L)	0.15 ± 0.07	0.16 ± 0.09	>0.05
TAC (mmol Trolox equiv./L)	0.98 ± 0.13	0.99 ± 0.14	>0.05

BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ox-LDL, oxided low-density lipoprotein; lipid hydroperoxide; TOS, total oxidant status; OSI, oxidative stress index; SH, free sulfhydryl groups (total thiol); TAC, total antioxidant capacity

*Measurements are given as the mean ± SD.

grade 3 ($n = 49$), and grade 4 ($n = 50$). The lowest and highest mean ox-LDL levels were observed in patients with grades 1 and 4, respectively (ANOVA, $P < 0.001$, Fig. 1). The relationships between ox-LDL and clinical and laboratory characteristics are demonstrated in Table 2. A correlation was established between the ox-LDL and radiographic grading and PON1 ($p < 0.001$ for all). In addition, there was an association between the ox-LDL and oxidative stress, age, and WOMAC score ($p < 0.05$ for all; Table 2). The inverse correlation between serum ox-LDL and PON1 is shown in Fig. 2. Also, the ox-LDL was independently associated with OA grade ($\beta = 0.440$, $p < 0.001$) and age ($\beta = 0.211$, $p = 0.002$) according to a multiple regression analysis (Table 2).

Discussion

In previous studies, it has been shown that ox-LDL may be associated with the pathogenesis of OA in vitro. However, the association between serum ox-LDL, oxidative stress, and PON1 in OA is unknown, and we believe that, if there is an association between serum ox-LDL, oxidative stress and PON1, ox-LDL may be a biomarker for follow-up in patients with knee OA. The present study showed that ox-LDL and oxidative stress parameters were significantly higher in an OA versus control group, whereas PON1 was significantly lower in the OA group. In addition, serum ox-LDL was positively correlated with oxidant parameters, knee OA grade, and
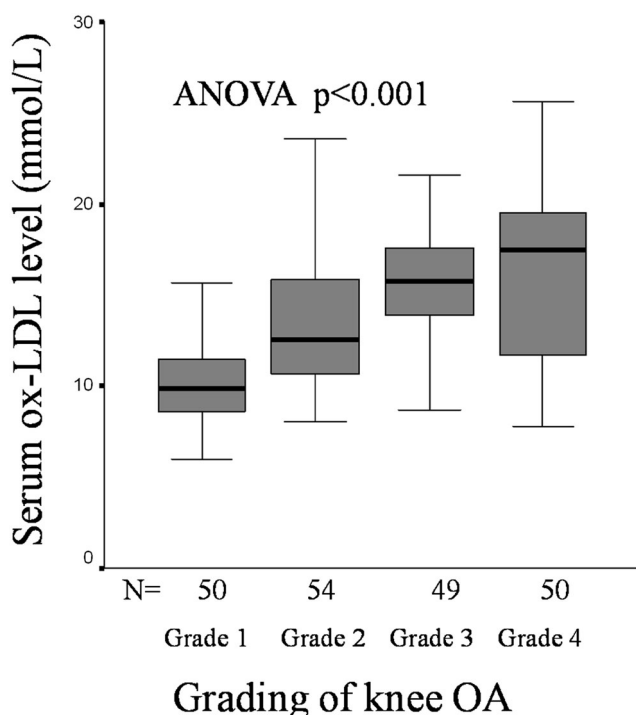

Fig. 1 Lowest and highest mean serum oxidized low-density lipoprotein (ox-LDL) levels were detected in patients with grades 1 and 4, respectively (ANOVA, $p < 0.001$)

WOMAC score, but inversely correlated with serum PON1. Finally, ox-LDL was positively correlated with radiographic severity and WOMAC score.

We found that serum ox-LDL was significantly higher in patients with OA. The increased serum ox-LDL in patients with knee OA could be due to oxidative stress. This hypothesis is supported by a previous study by Nishimura et al. [4], who showed that ox-LDL increases the production of intracellular ROS via activation of NF-kappaB in cultured bovine articular chondrocytes . [4]. It is known that a higher plasma level of ox-LDL is a risk factor for atherosclerosis, which is important in the development and progression of cardiovascular disease and its complications [6, 7, 24]. ox-LDL was also produced by generation of ROS from different cell types such as endothelial cells, vascular smooth muscle cells, leukocytes, and platelets [25–27]. On the other hand, there are similarities between OA and atherosclerosis [9, 28]. OA, similar to atherosclerosis, is an age-related inflammatory disease caused by inflammatory mediators released by cartilage, bone, and the synovium [29]. In addition, as in atherosclerosis, altered lipid metabolism has been implicated as playing a critical role in OA development [9, 28, 30, 31]. Increased lipid content in the superficial layer of articular cartilage has been reported [32]. Furthermore, synovial tissue in joints with OA is often inflamed, and inflammatory cytokines, including ox-LDL – which is oxidatively modified extra-articularly – contribute to cartilage damage [33]. Therefore, inflammatory cells in articular joints secrete ROS, which could promote the oxidation of natural LDL to ox-LDL [4, 5].

In the current study, levels of oxidative stress markers were significantly higher in OA. These results confirm that OA is associated with oxidative damage, in agreement with previous studies [11, 23]. We believe that LOOH can be used as a predictor of oxidative stress. LOOH is the first comparatively stable product of the lipid peroxidation reaction and is an important tool in oxidative stress research [22, 34, 35]. In addition, ox-LDL, as the basic structure of LOOH, is closely associated with the atherosclerotic process [34]. Likewise, lipid peroxidation to cartilage matrix protein degradation has been clearly demonstrated in the etiopathogenesis of OA [1]. Furthermore, in the current study, serum ox-LDL was positively correlated with oxidative stress markers. Collectively, these results suggest that the cause of elevated serum ox-LDL levels may be increased lipid oxidation.

In our previous study [11], we showed important relationships between decreased serum PON1 and increased oxidative stress parameters and knee OA. Therefore, we explored the correlation between serum PON1 and ox-LDL in knee OA. Our study showed that the ox-LDL level was inversely correlated with PON1 in knee OA. These results confirm the relationship between PON1 and its protective role against LDL oxidation [15, 16]. PON1 is an important component of the antioxidant mechanism. Indeed, it is known to protect LDL

Table 2 Relationships between serum ox-LDL level and clinical characteristics and laboratory parameters

	Pearson's correlation coefficient	p value	β regression coefficient*	p value
Grading of knee OA	0.502	<0.001	0.440	P < 0.001
Paraoxanase activity (U/L)	−0.337	<0.001		
LOOH (µmol/L)	0.214	=0.002		
TOS (µmol H$_2$O$_2$ equiv./L)	0.235	=0.001		
OSI (arbitrary units)	0.208	=0.003		
Age (years)	0.272	<0.001	0.211	p = 0.002
WOMAC score	0.255	<0.001		

*From multiple linear regression.

OA, osteoarthritis; LOOH, lipid hydroperoxide; TOS, total oxidant status; OSI, oxidative stress index; WOMAC, Western Ontario and McMaster University Osteoarthritis Index.

against oxidation stimulated either by copper ion or by a free radical generator system [15, 36].

A correlation was established between the ox-LDL level and radiographic severity. The highest ox-LDL levels were observed in grade 4 OA, with the lowest levels seen in grade 1 OA. The cause of the increase in ox-LDL with advancing OA stage is unknown, but structural changes are advanced in an 'end-stage' knee joint. Similarly, Akagi et al. [37] reported that the immunoreactivity of ox-LDL was correlated with the grade of cartilage degradation in OA. Therefore, ox-LDL can lead to local inflammation throughout the joint and accelerate the pathophysiology of cartilage degradation [9, 28]. Furthermore, there was an association between ox-LDL and WOMAC score, possibly due to oxidation of lipids under increased oxidative stress in OA. The symptoms in knee OA

are well-described by the WOMAC score [20]. The altered histological structure of the articular cartilage, synovial tissue, joint capsule, and other soft tissues may be responsible for joint pain and symptoms [38].

In this study, we have established a relationship between PON-I and ox-LDL and other lipo-proteins in different grades of OA. However, establishing these as markers need further studies as both PON-I and ox-LDL show a similar trend in other inflammatory diseases as rheumatoid arthritis.

Clinical implications

Notably, there is an important interconnection between lipid metabolism (body fat mass) and knee OA [39, 40]. Therefore, in the elderly, to increase muscle mass, physical activity should be encouraged. In addition, statins – which are widely used for the treatment of hypercholesterolemia – may also limit the progression of OA [41]. Furthermore, antioxidant treatments with nutraceuticals may prevent or alleviate OA as adjuvants [39].

There were some limitations to this study. First, it used a cross-sectional design. Second, serological correlation studies could potentially provide greater insights into the mechanisms of knee OA. In addition, measurements in synovial fluid could support our results. However, we did not have the opportunity to perform synovial aspiration for ethical reasons.

Conclusions

In conclusion, we found an association between the levels of various serum markers of oxidative injury, especially ox-LDL, and increasing severity of knee OA, as well as indirect evidence for their regulation by PON1. It is possible that increased oxLDL levels are associated with oxidative stress, as observed in cardiovascular disease. oxLDL seems to play a critical role in OA, both in the beginning, and during
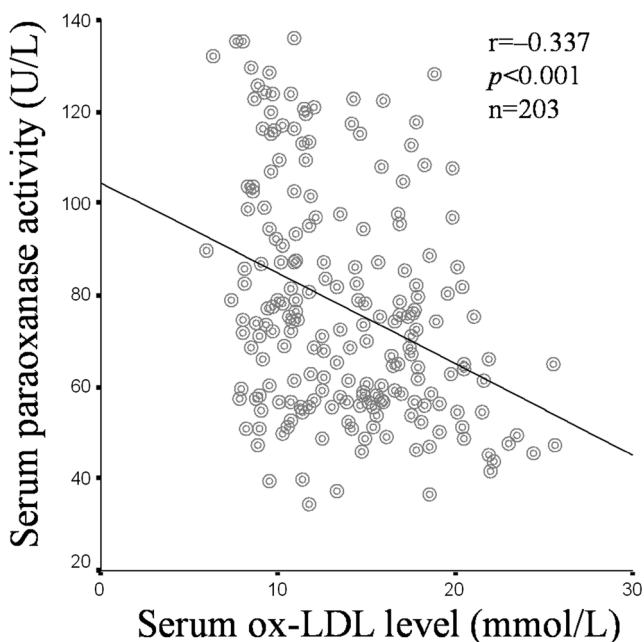

r=−0.337
p<0.001
n=203

Fig. 2 Correlation between serum paraoxonase activity and ox-LDL levels in knee osteoarthritis

progression of, the disease. Therefore, serum oxLDL levels may be a helpful biomarker to evaluate the severity of knee OA. The information presented here in represents an important contribution to the field.

Compliance with ethical standards

Disclosures None

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