Association of Plasma Marker of Oxidized Lipid with Histologic Plaque Instability in Patients with Peripheral Artery Disease

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Background: The association between oxidized low-density lipoprotein (OxLDL) and plaque instability in coronary and carotid artery disease is well established. However, the association between OxLDL and the histologic changes of plaque in peripheral artery disease has not been clearly elucidated. This study aims to investigate the association between plasma OxLDL and histologic plaque instability in patients with peripheral artery disease.

Methods: Prospectively obtained plaques from 48 patients who underwent endovascular atherectomy ($n = 20$), surgical endarterectomy ($n = 9$), or bypass surgery ($n = 19$) for treatment of atherosclerotic femoropopliteal artery disease were evaluated for histologic fibrosis, sclerosis, calcification, necrosis, cholesterol cleft, and foamy macrophages using hematoxylin and eosin, oil red O, and immunohistochemical staining. Unstable plaques were defined as plaques that were positive for foamy macrophages and with lipid content of more than 10% of the total plaque area. Plasma OxLDL levels were measured using an enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden).

Results: Of the 48 patients, 26 (54%) had unstable plaques. The unstable plaque group was younger, had fewer angiographic total occlusions, less calcification, and more CD68-positive and LOX-1-positive cells than the stable plaque group. Plasma OxLDL levels were significantly higher in the unstable plaque group than in the stable plaque group (57.4 ± 13.9 vs. 47.2 ± 13.6 U/L, $P = 0.014$). Multivariate analysis revealed that plasma OxLDL level, smoking, angiographic nontotal occlusion, and statin nonuse were independent predictors of unstable plaque.

Conclusions: Among patients with peripheral artery disease, the histologic instability of femoropopliteal plaque was independently associated with high plasma OxLDL, smoking, nontotal occlusion, and statin nonuse. Further large-scale studies are necessary to evaluate the role of noninvasive OxLDL measurement for predicting plaque instability and future adverse vascular event.

Kichun Kim and Cheong Lim contributed equally to this manuscript.

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INTRODUCTION

Atherosclerosis is a chronic progressive disease that involves vascular endothelial dysfunction, lipid infiltration, foam cell formation, and vascular smooth muscle cell proliferation.[1–4] In atherosclerosis, the subendothelial retention of low-density lipoproteins and their oxidative modification play a critical role. Oxidized low-density lipoproteins (OxLDLs) are considered one of the key factors in the initiation and acceleration of atherosclerosis.[5,6]

In a previous study, plasma OxLDL levels were significantly correlated with coronary plaque instability and the severity of acute coronary syndromes.[7] The association between OxLDLs and the instability of carotid plaque[8] and ultrasonographic morphology of common femoral plaque[9] has also been reported.

As the role of OxLDLs in atherosclerosis has been well established, there have been many studies focusing on how OxLDLs can act as predictors of cardiovascular events. For instance, studies have shown a positive correlation between serum OxLDL levels and the incidence of future cardiovascular events.[10–13] Additionally, in an ultrasound study regarding plaque ruptures, patients with ruptured plaque in their iliofemoral arteries had higher incidence rates of cardiac, cerebrovascular, and peripheral vascular events than patients without ruptured plaque.[14] A recent study has also shown that plaques with lipid-rich necrotic cores in the superficial femoral artery are associated with higher rates of peripheral artery disease (PAD) events.[15]

Currently, most OxLDL studies are focused on coronary and carotid artery disease, and relatively few OxLDL studies have been done in relation to PAD. We aimed to investigate the relationship between plasma OxLDLs and plaque instability via the histologic evaluation of PAD patients.

METHODS

Study Subjects

We prospectively enrolled 48 patients with PAD who underwent percutaneous transluminal angioplasty (PTA) with endovascular atherectomy, surgical endarterectomy, or bypass surgery, for treatment of at least 50% diameter stenosis of the femoropopliteal artery, at 2 South Korean university hospitals, Seoul National University Bundang Hospital and Seoul National University-Seoul Metropolitan Government Boramae Medical Center, between May 2012 and November 2016. Indications for revascularizations include claudication, resting limb pain, or tissue loss not relieved by exercise therapy and medical treatment, decreased ankle-brachial index, and/or significant narrowing or occlusion in noninvasive imaging studies such as Doppler ultrasound or computed tomographic angiography. Patients younger than 15 years, older than 90 years, those already participating in other clinical trials, and those with restenosis lesions and lesions caused by disorders other than atherosclerosis, such as vasculitis, infection, or embolization, were excluded from the study. Information regarding patients' baseline characteristics and patients' laboratory and angiographic findings were collected from the electronic medical records from each hospital.

Written informed consent was obtained from each participant before PTA and surgery. Our study protocol was approved by the Institutional Review Board of each hospital. The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

Endovascular and Surgical Techniques

Endovascular atherectomy was performed in a standard manner with the SilverHawk™ catheter (SilverHawk peripheral plaque excision system, Medtronic, Plymouth, Minnesota, USA). A 7F guiding catheter via the common femoral artery and 0.014-inch guidewire were used for the procedure. Unfractionated heparin (100 IU/kg) Q1 was administered intravenously before the insertion of the guidewire. The SilverHawk LX-M was used for the common femoral artery and the proximal superficial femoral artery in most patients, while the SilverHawk MX-M was used for the distal superficial femoral artery and the popliteal artery. The first run of atherectomy was started in the direction of angiography showing most significant stenosis and with the 90° rotation of the angiographic angle between each run; total 2 to 4 runs of atherectomy were performed for a lesion to remove plaque until a visually estimated residual diameter stenosis of less than 30% was achieved or when significant dissection occurred.

Surgical endarterectomy and bypass surgeries were performed in standard fashion. For the endarterectomy, after the common femoral artery was fully dissected with minimal manipulation to avoid plaque disruption or distal embolization, intravenous unfractionated heparin (100 IU/kg) Q1 was administered before clamping, and the activated coagulation time was maintained at over 200 sec. The intraluminal atheroma was retrieved using a dissector by gently peeling away along the sub-intimal plane, leaving the adventitial layer intact. Thereafter, the arterial lumen was cleaned using a heparinized normal saline

solution. Arteriotomy incision was augmented using a bovine pericardial strip after endarterectomy. Bypass surgeries were performed using either graft made of enhanced poly-tetrafluoroethylene or autologous saphenous vein when appropriate. After careful selection of inflow and outflow site, graft anastomosis was usually performed with monofilament polypropylene sutures in continuous running or simple interrupted manners by each surgeon's preference. Specimens were obtained in the same manner as the surgical endarterectomy.

Measurement of Plasma OxLDL Levels

I total, 10 mL blood samples were taken from each patient via arterial sheaths at the end of PTA and surgery and were stored in heparin tubes. Plasma was separated from blood samples via centrifugation at 3,000 rpm for 10 min at 4°C. Then, 0.5 μL of 40 mg/mL butylated hydroxytoluene (Sigma-Aldrich, St. Louis, USA) and 50 μL of 10 mg/mL glutathione (Sigma-Aldrich, St. Louis, USA) were added to 500 μL of plasma to prevent in vitro oxidation during the process of sample preparation, and stored at −80°C. Plasma OxLDL levels were measured using an enzyme-linked immunosorbent assay (MercodiaAB, Uppsala, Sweden).

Specimen Collection

Atherosclerotic plaque specimens obtained during PTA or surgery were transferred to the laboratory within 1 hr and processed. Specimens from a lesion were divided into 3 pieces and stored for histologic analysis. Among several atherectomy specimens collected from a lesion, 3 largest specimens were selected for the analysis. The location of the lesion was identified from the angiographic images performed during each run of atherectomy. Regarding the orientation, in general, the largest specimen was obtained from the first run of atherectomy in the orientation of the most severe stenosis. Among the 3 specimens, the largest one was fixed using 10% formalin, another one was freshly frozen at −80°C, and the last piece was fixed using an optimal cutting temperature compound and stored at −80°C. Formalin-fixed samples were cut into 4 μm thickness and used for hematoxylin and eosin (H&E) staining and immunohistochemical staining, whereas the fresh-frozen samples were processed as a tissue section of 5 μm thickness and then stained using the oil red O stain.

Histologic Assessment

H&E staining was performed to evaluate each specimen for fibrosis, sclerosis, calcification, necrosis, and cholesterol clefts. Oil red O staining was performed to analyze specimens' lipid contents. Immunohistochemical analysis was performed using anti-CD68 (1:200, clone KP1, Thermo Fisher Scientific, Fremont, CA, USA) for identifying macrophages, anti-lectin-like OxLDL receptor-1 (LOX-1) (1:2000, ab60178, Abcam, Cambridge, UK) for identifying OxLDL receptors on macrophages (Fig. 1), and anti-smooth muscle actin (1:500, 1A4, Cell Marque, Rocklin, CA, USA) for identifying vascular smooth muscle cells. Histologic examinations were performed by 2 experienced pathologists (GK and JHC). Among multiple slide sections for each stain from a patient, one slide section with the largest and most obvious plaque was used for histologic examination. Each component of histologic examination was measured as a percentage of the total plaque area on the slide section. Unstable plaques were defined as plaques with a lipid content of more than 10% in the oil red O staining and with the presence of foamy macrophages.[8,16,17]

Statistical Analysis

Continuous variables were expressed as ''mean ± standard deviation'' and medians with interquartile ranges. Categorical data were summarized as frequencies and proportions. Continuous variables were compared using Student's *t*-test (parametric method) and the Mann−Whitney *U* test (nonparametric method). Categorical variables were compared using chi-square tests and Fisher's exact test when any cell count was less than 5 in a 2 × 2 contingency table. A logistic regression model was applied to verify the predictors of unstable plaque. Variables found to be statistically significant in the univariable analyses and factors already known to be risk factors for PAD[18] were included in the multivariable model. Variables that were closely related to other clinical variables were excluded from the multivariable model due to possible multicollinearity. All statistical tests were two-sided. A P of less than 0.05 was considered statistically significant. SPSS 22.0 software (SPSS, Inc., IBM, Chicago, IL, USA) was used for our analyses.

RESULTS

Baseline Characteristics

Study patients' mean age was 70 years, and 77.1% of patients were male (Table I). More than a third of our patients had a Rutherford grade of 4 or more symptom, 70.8% had diabetes mellitus (DM), 43.8% were current smokers, 83.3% were

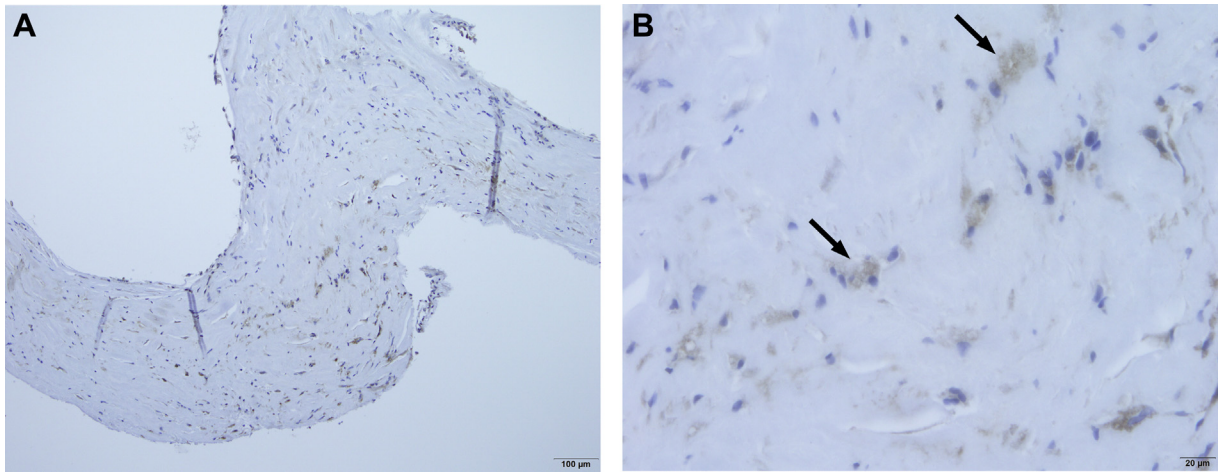

Fig. 1. Representative histologic image of LOX-1 expression in plaque. Immunohistochemical staining of plaque showing accumulation of LOX-1 proteins on present macrophages (*arrows* in **B**) (**A**, magnification ×100) (**B**, magnification ×400). LOX-1, lectin-like oxidized low-density lipoproteins receptor-1.

taking statin, and angiographic total occlusion was confirmed in 54.2% of patients. In terms of revascularization treatment, 41.6% of the patients underwent endovascular atherectomy, 18.8% underwent surgical endarterectomy, and 39.6% underwent bypass surgery. More than half (56.3%) of the plaque specimens were obtained from the common femoral artery. One to three specimens were collected from each patient. The average mass of specimen per patient was 105 mg.

Of the 48 patients, 26 (54.2%) patients were classified into the unstable plaque group and the rest were classified into the stable plaque group (Table I). The unstable plaque group was significantly younger (66.9 ± 9.7 versus 73.8 ± 7.4 years, $P = 0.009$); had significantly fewer total occlusions (38.5 versus 72.7%, $P = 0.018$) and less calcification (21.1 versus 76.9%, $P = 0.002$); and more prevalent in specimens from endovascular atherectomy procedure than the stable plaque group (55.7 versus 22.7%, $P = 0.014$). There were no statistically significant differences between the groups in terms of other medical history, laboratory tests, and medication at admission.

Oxidized Lipid Marker and Histologic Findings

Plasma OxLDL levels of the entire study population showed normal distribution (Kolmogorov−Smirnov test Z = 0.087) and were significantly higher in the unstable plaque group than in the stable plaque group (57.4 ± 13.9 versus 47.2 ± 13.6 U/L, $P = 0.014$) (Fig. 2A). Immunohistochemical staining using anti-LOX-1 and anti-CD68 also showed significantly higher LOX-1 (56.2 ± 38.3 versus 17.7 ± 37.5%, $P = 0.002$) and CD68 (71.9 ± 38.3 versus 14.1 ± 35.0%, $P < 0.001$) expression rates, respectively, in unstable plaques than in stable plaques (Fig. 2B) (Table II). Furthermore, there was less calcification in the unstable plaque group than in the stable plaque group (4.2 ± 6.1 versus 20.9 ± 25.5%, $P = 0.006$), but 2 groups did not differ significantly from each other in terms of other histologic findings. Representative histologic examples of unstable and stable plaque are shown in Figure 3.

Risk Factors for Unstable Plaque

The univariate analysis showed that younger ages, higher plasma OxLDL levels, fewer angiographic total occlusions, lower degrees of stenosis, smaller amounts of calcification, lower transatlantic intersociety consensus lesion classifications, and specimens from endovascular or surgical atherectomies were significantly associated with unstable plaque (Table III). The multivariate analysis revealed that smoking (odds ratio [OR] = 10.207; 95% confidence interval [CI], 1.480−70.230; $P = 0.018$) and high plasma OxLDL levels (OR = 1.090; 95% CI, 1.021−1.165; $P = 0.010$) were significant independent positive predictors of unstable plaque, whereas angiographic total occlusion (OR = 0.044; 95% CI, 0.006−0.323; $P = 0.002$) and the baseline statin use (OR = 0.099; 95% CI, 0.011−0.867; $P = 0.037$) were significant independent negative predictors of unstable plaque.

Table I. Baseline characteristics of study subjects

Characteristic	Total ($n = 48$)	Unstable plaque group ($n = 26$)	Stable plaque group ($n = 22$)	*P*
Age, year	70.1 ± 9.3	66.9 ± 9.7	73.8 ± 7.4	0.009
Sex, male, *n* (%)	37 (77.1)	19 (73.1)	18 (81.8)	0.473
Weight, kg	62.5 ± 11.3	62.0 ± 12.0	63.1 ± 10.6	0.986
BMI, kg/m^2	23.6 ± 4.0	23.5 ± 4.6	23.8 ± 3.4	0.778
Rutherford grading, *n* (%)				0.454
0 (no symptom)	1 (2.1)	1 (3.8)	0 (0.0)	
1−3 (claudication)	29 (62.1)	14 (53.8)	15 (68.2)	
4 (resting pain)	12 (25)	6 (23.1)	6 (27.3)	
5 (minor tissue loss)	6 (12.5)	5 (19.2)	1 (4.5)	
6 (major tissue loss)	0 (0.0)	0 (0.0)	0 (0.0)	
Critical limb ischemia, *n* (%)	18 (37.5)	11 (42.3)	7 (31.8)	0.454
Symptom onset, remote, month	31.2 ± 44.8	33.2 ± 45.4	29.0 ± 45.2	0.765
Symptom onset, recent, month	11.6 ± 23.6	11.5 ± 20.6	11.3 ± 26.9	0.979
Past medical history, *n* (%)				
Diabetes mellitus	34 (70.8)	18 (69.2)	15 (68.2)	0.938
Duration of DM, year	12.0 ± 12.3	12.2 ± 10.1	12.6 ± 14.8	0.929
Use of insulin	19 (57.6)	11 (61.1)	8 (53.3)	0.653
Dyslipidemia	32 (66.7)	16 (61.5)	16 (72.7)	0.413
Current smoking	21 (43.8)	14 (53.8)	7 (31.8)	0.304
ESRD on dialysis	6 (12.5)	4 (15.4)	2 (9.1)	0.511
Acute coronary syndrome	14 (29.2)	6 (23.1)	8 (36.4)	0.313
Myocardial infarction	4 (8.3)	2 (7.7)	2 (9.1)	0.861
Cerebrovascular disease	11 (22.9)	4 (15.4)	6 (27.3)	0.509
Prior endovascular revascularization of PAD	10 (20.8)	4 (15.4)	6 (27.3)	0.478
Prior surgical revascularization of PAD	3 (6.3)	1 (3.8)	2 (9.1)	0.587
Laboratory findings				
BUN, mg/dL	44 (91.7)	25 (96.2)	19 (86.4)	0.221
Creatinine, mg/dL	1.1 ± 1.0	1.1 ± 1.4	1.0 ± 0.7	0.755
eGFR, ml/min/m^2	66.0 ± 36.5	58.8 ± 34.1	74.5 ± 38.2	0.139
Total cholesterol, mg/dL	152.3 ± 38.7	160.2 ± 40.4	142.9 ± 35.1	0.123
Triglyceride, mg/dL	146.0 ± 68.6	148.1 ± 74.6	142.9 ± 61.0	0.831
HDL cholesterol, mg/dL	39.0 ± 8.0	39.0 ± 12.0	39.5 ± 10.0	0.309
LDL cholesterol, mg/dL	84.8 ± 33.2	91.2 ± 36.3	75.2 ± 26.3	0.165
HbA1C, %	7.3 ± 1.2	7.5 ± 1.6	7.0 ± 0.7	0.270
hs-CRP, mg/dL	1.7 ± 2.9	1.7 ± 3.3	1.7 ± 2.4	0.921
Urine microalbumin/creatinine ratio	59.1 ± 1,133.8	75.3 ± 1,122.7	31.0 ± 1,147.3	0.691
Ankle-brachial index	0.6 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	0.163
Medication at admission[a], *n* (%)				
Aspirin	38 (79.2)	19 (73.1)	19 (86.4)	0.259
Clopidogrel	21 (43.8)	11 (42.3)	10 (45.5)	0.827
Anti-coagulant	2 (4.2)	1 (3.8)	1 (4.5)	0.904
Cilostazol	13 (27.1)	10 (38.5)	9 (40.9)	0.863
Statin	40 (83.3)	16 (61.5)	18 (81.8)	0.124
Angiographic findings				
Lesion length, cm	12.5 ± 11.9	10.0 ± 0.3	15.4 ± 13.2	0.136
Angiographic stenosis (%)	93.8 ± 8.9	91.1 ± 9.5	97.1 ± 7.0	0.019
Total occlusion, *n* (%)	26 (54.2)	10 (38.5)	16 (72.7)	0.018
Moderate to severe calcification, *n* (%)	14 (43.8)	3 (21.1)	10 (76.9)	0.002
Thrombus on angiography, *n* (%)	6 (14.6)	2 (8.7)	4 (22.2)	0.263
TASC femoropopliteal classification (C or D)	22 (48.9)	8 (32.0)	15 (68.2)	0.083
Popliteal involvement, *n* (%)	6 (12.5)	3 (11.5)	3 (13.6)	0.827
Popliteal P2/P3 involvement, *n* (%)	5 (10.4)	3 (11.5)	2 (9.1)	0.782
Endovascular atherectomy, *n* (%)	20 (41.6)	15 (57.7)	5 (22.7)	0.014

(*Continued*)

Table I. Continued

Characteristic	Total ($n = 48$)	Unstable plaque group ($n = 26$)	Stable plaque group ($n = 22$)	*P*
Surgical revascularization, *n* (%)				
Endarterectomy	9 (18.8)	5 (19.2)	4 (18.2)	0.926
Bypass surgery with endarterectomy	4 (8.3)	1 (3.8)	3 (13.6)	0.221
Bypass surgery without endarterectomy	15 (31.3)	5 (19.2)	10 (45.5)	0.051
Plaque collection location, *n* (%)				0.058
Common femoral artery	27 (56.3)	11 (22.9)	16 (33.3)	
Proximal superficial femoral artery[b]	12 (25.0)	10 (20.8)	2 (4.2)	
Distal superficial femoral artery	8 (16.7)	5 (10.4)	3 (6.3)	
Popliteal artery	1 (2.1)	0 (0)	1 (4.5)	

Categorical variables are depicted as the number of patients (percentage); continuous variables are depicted as medians (interquartile ranges) or mean (standard deviations).

BMI, body mass index; DM, diabetes mellitus; ESRD, end-stage of renal disease; PAD, peripheral artery disease; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate by MDRD equation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; TASC, TransAtlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease.

[a]Medication taking for at least 6 months at the time of admission were included.

[b]The division point for proximal and distal superficial artery was the center of the entire superficial femoral artery.

DISCUSSION

Our results showed that high plasma OxLDL levels were significantly associated with histologically distinguished unstable plaques in lower extremity artery disease. To our knowledge, this is the first study to report the relationship between histologically verified instability of femoropopliteal artery plaque and plasma OxLDL levels. In addition, smoking, angiographic nontotal occlusion, and nonuse of statin were also significantly associated with histologic unstable plaque.

OxLDL and Plaque Instability

OxLDLs play key roles in the initiation and progression of atherosclerosis,[5,6] and previous studies have shown a strong association between plasma OxLDLs and plaque instability in coronary and carotid artery disease.[8,17,19] Our results are also consistent with those of the previous studies that report a relationship between OxLDLs and occurrence or instability of common femoral artery plaque.[9,20,21] Furthermore, while all the previous studies have estimated plaque instability by ultrasonographic echogenicity,[9,20,21] our study is meaningful in that plaque instability is directly assessed through histologic evaluation.

Plaque Instability and Future Vascular Events

In previous histologic plaque studies, coronary artery plaques with lipid-rich cores and thin fibrous caps were known to increase the risk of plaque rupture and cause the development of acute coronary syndrome and progression of coronary artery atherosclerosis.[22,23] It has also been reported that carotid and lower extremity artery plaques with lipid-rich cores are associated with ischemic stroke,[24,25] cardiovascular events,[26] and PAD events,[15] suggesting that unstable plaque is a strong risk factor for future vascular events. If plaque instability can be assessed with noninvasive measurement of serum OxLDL, the risk of future adverse vascular events may also be predicted, thereby allowing for the implementation of intensifying plaque stabilizing therapy with statins and antithrombotic agents, reinforcing the surveillance, and performing earlier percutaneous or surgical intervention. In this regard, we generate a hypothesis that plasma OxLDLs could be one of the predictive markers for plaque instability in patients with PAD.

Other Independent Predictors of Femoropopliteal Plaque Instability

Current smoking. Multivariate analysis of our study revealed that current smoking, angiographic nontotal occlusion, and nonuse of statins were independent predictors of unstable plaque in the femoropopliteal artery. A previous report on the autopsy sample of coronary plaque from patients of sudden death showed that plaque of the acute thrombosis group was more significantly associated with higher prevalence of smoking than stable plaque group.[27] Further, among patients with PAD, presence of MRI-detected high-risk superficial femoral artery plaque was independently predicted by current smoking,[28] showing similar results as -those of the current study.

Angiographic nontotal occlusion. In coronary artery disease, unstable plaques are usually called as

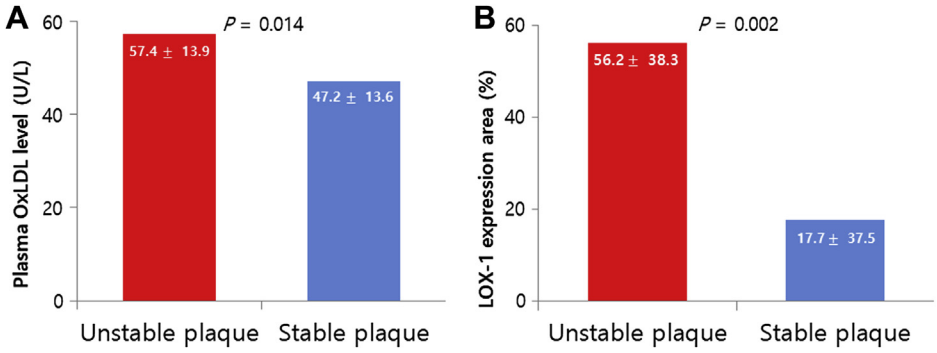

Fig. 2. Comparison of the plasma and histologic oxidized lipid markers between the unstable plaque group and the stable plaque group. The plasma OxLDL level is significantly higher in the unstable plaque group than that in the stable plaque group **(A)**. The immunohistochemical LOX-1 expression area is significantly greater in the unstable plaque group than that in the stable plaque group **(B)**. OxLDL, oxidized low-density lipoproteins; LOX-1, lectin-like oxidized low-density lipoproteinreceptor-1.

Table II. Histologic findings in unstable and stable plaque

Findings	Total (n = 48)	Unstable plaque (n = 26)	Stable plaque (n = 22)	P
Fibrosis (%)	56.6 ± 31.6	64.0 ± 23.1	47.7 ± 38.1	0.089
Sclerosis (%)	23.1 ± 21.7	21.7 ± 20.3	24.8 ± 23.6	0.633
Calcification (%)	11.9 ± 19.5	4.2 ± 6.1	20.9 ± 25.5	0.006
Necrosis (%)	2.3 ± 10.4	1.2 ± 3.6	3.6 ± 15.0	0.416
Cholesterol cleft (%)	2.4 ± 3.9	2.9 ± 3.8	1.8 ± 4.0	0.345
Oil red O (%)	43.8 ± 26.6	45.8 ± 22.8	41.4 ± 31.1	0.596
CD68 (%)	45.4 ± 46.6	71.9 ± 38.3	14.1 ± 35.0	<0.001
Smooth muscle actin (%)	85.9 ± 22.3	86.2 ± 16.8	85.5 ± 27.9	0.906
LOX-1 (%)	37.0 ± 42.2	56.2 ± 38.3	17.7 ± 37.5	0.002

LOX-1, lectin-like oxidized low-density lipoprotein receptor-1.
Fibrosis, sclerosis, calcification, necrosis, cholesterol cleft, and oil red O were measured as a percentage of the total plaque area. CD68 and LOX-1 were measured as the ratio of positive macrophage in total macrophage in an H&E stain. Smooth muscle actin was measured as the ratio of positive smooth muscle cell in total smooth muscle cell.

'vulnerable plaque,' characterizing the tendency for plaque disruption and thrombus formation,[29] which can result in acute coronary syndrome. Several angiographic studies showed that culprit lesions responsible for myocardial infarction develop from lesions with <50% diameter stenosis at baseline angiography.[30–32] In addition, it has been suggested that most myocardial infarctions result from ruptures of mildly stenotic plaques, and most adverse coronary events take place irrespective of the severity of baseline angiographic luminal stenosis.[33]

Furthermore, in a more recent, prospective large-scale study of patients with acute coronary syndrome, initial nonculprit lesions responsible for recurrent adverse events during follow-up were lesions with mild stenosis at baseline angiography.[34] In that prospective study, intravascular ultrasound imaging done at baseline revealed that large plaque burden and plaque morphology with thin cap and large lipid core were main predictors of adverse events.[34] Therefore, for coronary artery disease, features of vulnerable plaque could be evaluated by intravascular imaging, rather than by angiographic stenosis. One of the reasons for this discrepancy between angiographic stenosis and intravascular imaging is positive remodeling, which usually occurs during coronary plaque progression, and which can harbor high plaque burden without causing luminal stenosis.[35]

Also, in most large-scale lipid-lowering trials, there was much larger improvement in clinical adverse cardiac events without improvement in angiographic luminal stenosis.[36] Furthermore, in a coronary chronic total occlusion lesion, occluded segment is mainly a fibrous tissue,[37] and as the duration of occlusion increases, the component of the lesion changes, and there will be fewer macrophages and more fibrotic tissue,[38] showing histologic characteristics similar to those of a stable plaque.

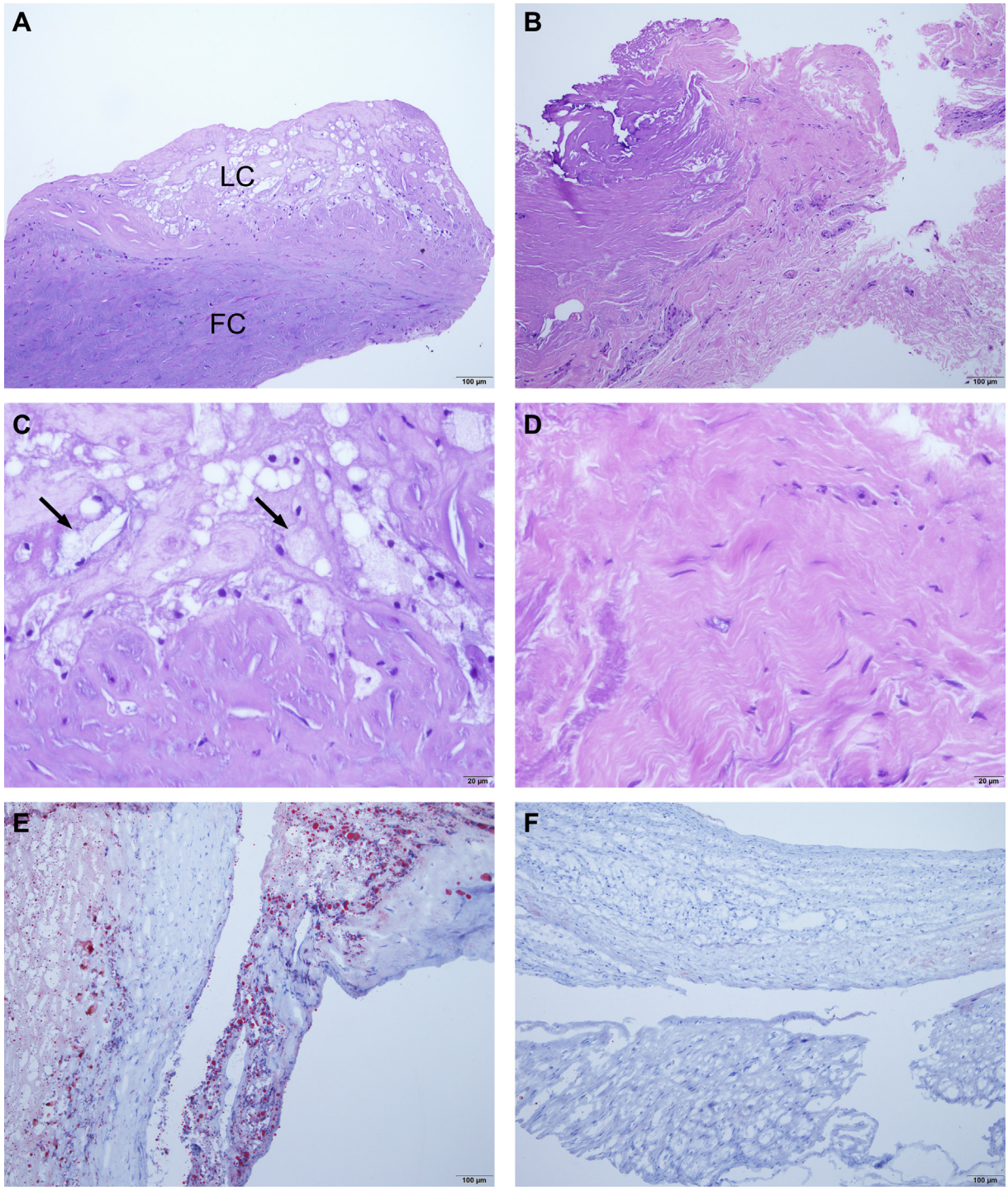

Fig. 3. Representative histologic example of unstable plaque **(A, C** and **E)** and stable plaque **(B, D** and **F)** in common femoral artery **(A, C** and **E)** and proximal superficial femoral artery **(B, D** and **F)**. Hematoxylin and eosin (H&E) stain showing the lipid-rich core (LC in **A**), thin fibrous cap (FC in **A**), and foamy macrophage (*arrows* in **C**) of unstable plaque (**A**, ×100 magnification; C, ×400 magnification, respectively).Thick fibrous tissue and a small amount of lipids in an H&E stain from a stable plaque sample (**B**, ×100 magnification; **D**, ×400 magnification, respectively). Oil red O stain shows the high density of lipid droplets in unstable plaque (**E**, ×100 magnification). Oil red O stain shows no lipid droplets in stable plaque (**F**, ×100 magnification).

Table III. Predictors for unstable plaque

	Univariate analysis		Multivariate analysis	
Risk factor	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, per year	0.903 (0.830−0.982)	0.018	0.964 (0.865−1.075)	0.511
Sex, male	0.603 (0.151−2.415)	0.475	0.523 (0.055−6.007)	0.643
BMI, per 1 kg/m^2	0.979 (0.849−1.129)	0.772		
Critical limb ischemia	0.747 (0.226−2.468)	0.632		
Symptom onset, recent, per month	1.000 (0.975−1.026)	0.979		
Hypertension	3.947 (0.380−3.947)	0.250		
Diabetes mellitus	1.050 (0.309−3.571)	0.952	0.931 (0.068−12.720)	0.957
Use of insulin	0.727 (0.181−2.914)	0.653		
Duration of DM, per year	0.997 (0.945−1.053)	0.926		
Dyslipidemia	0.600 (0.176−2.046)	0.414		
Acute coronary syndrome	0.747 (0.226−2.468)	0.632		
ESRD on dialysis	1.818 (0.300−11.023)	0.516		
Current smoking	2.500 (0.766−8.160)	0.152	10.207 (1.480−70.230)	0.018
eGFR, per 1 mL/min/m^2	0.988 (0.971−1.004)	0.141	0.998 (0.964−1.032)	0.889
Total cholesterol, per 1 mg/dL	1.013 (0.996−1.030)	0.129	1.003 (0.970−1.038)	0.853
HDL cholesterol, per 1 mg/dL	0.947 (0.873−1.027)	0.189		
LDL cholesterol, per 1 mg/dL	1.016 (0.993−1.040)	0.168		
OxLDL, per 1 U/L	1.058 (1.008−1.111)	0.023	1.090 (1.021−1.165)	0.010
hs-CRP, per 1 mg/dL	1.011 (0.823−1.241)	0.919		
Ankle-brachial index, per 0.1	5.614 (0.466−67.637)	0.174		
Urine albumin/creatinine ratio, per 1 mg/g	1.061 (0.752−1.497)	0.734		
Aspirin at admission	2.333 (0.523−10.400)	0.266		
Clopidogrel at admission	1.136 (0.362−3.569)	0.827		
Statin at admission	2.821 (0.736−10.751)	0.131	0.099 (0.011−0.867)	0.037
Angiographic total occlusion	0.234 (0.069−0.799)	0.020	0.044 (0.006−0.323)	0.002
Angiographic stenosis	0.907 (0.829−0.992)	0.033		
Angiographic calcification	0.080 (0.015−0.437)	0.004		
Thrombus on angiography	0.185 (0.017−2.024)	0.167		
TASC femoropopliteal classification (C or D)	0.220 (0.064−0.751)	0.016		
Endovascular or surgical atherectomy	3.256 (1.309−8.423)	0.011	1.752 (0.100−30.803)	0.702

Logistic regression model with backward elimination: included variables were age, sex, diabetes mellitus, current smoking, eGFR, total cholesterol, use of statin at admission, and those with $P < 0.05$ in univariate analysis.
Variables included in the multivariable analysis were age, sex, diabetes mellitus, current smoking, eGFR, total cholesterol, OxLDL, angiographic total occlusion, use of statin at admission, and undergoing endovascular revascularization.
OR, odds ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus; ESRD, end-stage of renal disease; eGFR, estimated glomerular filtration rate by MDRD equation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OxLDL, oxidized low-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; hs-CRP, high-sensitivity C-reactive protein; TASC, TransAtlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease.

Also, in PAD, in a pathology study with patients with critical limb ischemia, most of chronic thrombi were present in lesions with insignificant stenosis.[39] By contrast, in carotid artery disease, the correlation between stenosis severity and future risk of stroke is well established.[40]

Nonuse of statin. Regarding statin treatment, an autopsy study showed significantly higher total cholesterol level in the acute coronary thrombosis group than that in the stable plaque group.[27,41,42]

In intracoronary imaging studies, the use of statin was associated with less plaque rupture,[43] decreased macrophage infiltration,[44] reduced lipid content,[45] and increased fibrous component in the plaque,[46] suggesting a plaque stabilizing effect of statins. In a sub-analysis of a large, prospective, randomized trial of statin treatment, in patients with PAD, the use of statins reduced peripheral vascular events, irrespective of baseline cholesterol levels and other factors.[47]

However, in an MRI study of patients with PAD, statin nonuse did not result in an independent predictor of high-risk plaque, showing a conflicting result to that obtained in our study.[28]

Factors Showing Insignificant Differences between the Unstable Plaque Group and the Stable Plaque Group

In our study, in agreement with other large-scale studies regarding the nonsignificant relationship between the instability of the iliofemoral and carotid[48,49] artery plaque and DM, DM was not an independent risk factor of unstable plaque. Moreover, C-reactive protein, which is previously known to be associated with the presence of coronary and carotid plaque, but not with plaque morphology,[50,51] was not significantly associated with instability of femoropopliteal plaque in our study, either.

The relationship between symptom severity and arterial plaque instability has been seen in patients with acute coronary syndrome[52] and symptomatic carotid artery disease patients.[53] However, there have been no reports regarding the relationship between symptom severity and plaque instability in PAD patients. Our study showed that there were no significant differences in the PAD symptom severity grades or clinical presentation between the unstable plaque group and the stable plaque group. Although it has not been clearly elucidated, we cautiously speculate that symptom development due to plaque instability might be more blunted or much slower in PAD than that in coronary or carotid artery disease.

Limitations

This study has a few limitations. Firstly, selection bias may affect our results because, among the endovascular revascularization subjects, only patients who underwent atherectomy were included. However, more than half of the study population consisted of consecutive patients who underwent surgical revascularization, and this might have decreased the degree of selection bias. Secondly, our sample size is relatively small. Consequently, substantial overlap was observed in OxLDL levels in the unstable and stable plaque groups. However, we enrolled consecutive patients from 2 referral hospitals that were affiliated with the same university, without changing the clinical practice policy, thereby giving the study population more homogeneity.

CONCLUSIONS

In this study, plasma OxLDL levels showed a significant association with the histologic instability of femoropopliteal artery plaque. Moreover, smoking, nontotal occlusion, and statin nonuse were also significantly associated with unstable plaque. The results of our study suggest that high OxLDL could be one of noninvasive predictors of unstable plaque in patients with PAD; however, this requires further validation through large-scale studies.

E.J. Kim performed the laboratory experiments. H.Y. Shin and H.A. Heo reviewed the medical records.

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