The European Research Journal 2022;8(2):169-174 Original Article

DOI: 10.18621/eurj.870716 *Nephrology*

Carotid atherosclerosis and lectin-like oxidized low density lipoprotein receptor-1 levels in hemodialysis patients

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ABSTRACT

Objectives: Atherosclerotic cardiovascular disease risk is increased in hemodialysis patients. Oxidized low density lipoprotein has an important role in atherosclerotic process and it exerts this effect via lectin like oxidized low density lipoprotein-1 (LOX-1). Carotid artery intima-media thickness (CIMT) is accepted as a god indicator of subclinical atherosclerosis. In this study, we aimed to investigate LOX-1 and CIMT levels in hemodialysis patients.

Methods: Twenty-eight patients treated with hemodialysis at least 6 months and 19 healthy subjects were enrolled in this study. Serum LOX-1 levels and simultaneously with CIMT were measured in hemodialysis patients and healthy control group.

Results: CIMT value was found to be statistically significantly higher in the hemodialysis group compared to control group (0.9 mm in hemodialysis group vs. 0.7 mm in control group, $p < 0.001$). There was no statistically significant difference between groups in terms of LOX-1 levels. (0.172 ng/ml in hemodialysis group vs. 0.213 ng/ml in healthy control group, $p > 0.05$).

Conclusions: Although cardiovascular risk markers like CIMT, CRP were higher in hemodialysis group as expected, increase in LOX-1 levels was not detected.

Keywords: Atherosclerosis, Carotid intima-media thickness, Hemodialysis, Lectin-like oxidized low densitiy lipoprotein receptor-1

Cardiovascular diseases constitute the major cause of morbidity and mortality in patients with chronic kidney failure [1, 2]. Even when variables such as age, gender, and the presence of diabetes mellitus are adjusted, cardiovascular mortality is still 10-20 times higher in these patients compared to the normal population [3]. In chronic kidney disease, traditional cardiovascular risk factors alone are inadequate to explain the increase in cardiovascular mortality. Some unconventional risk factors associated with uremia are thought to play an important role in the development of atherosclerosis in these patients [4, 5]. Non-invasive sensitive indicators are needed to clarify these mechanisms and to recognize cardiovascular complications early.

Atherogenesis; which plays an important role in the pathophysiology of cardiovascular diseases, is characterized by the accumulation of plasma lipids, fibrous tissue, and cell components comprising mostly macrophages, smooth muscle cells, and lymphocytes

***Received:** January 30, 2021; **Accepted:** June 28, 2021; **Published Online:** January 19, 2022*

***How to cite this article:** Dağel T, Gönenli MG, Kahvecioğlu S. Carotid atherosclerosis and lectin-like oxidized low density lipoprotein receptor-1 levels in hemodialysis patients. Eur Res J 2022;8(2):169-174. DOI: 10.18621/eurj.870716*

e-ISSN: 2149-3189

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in large arteries. Traditional risk factors for atherogenesis include age, gender, diabetes mellitus, hypercholesterolemia, and smoking. Currently, high plasma and tissue levels of oxidized low-density lipoprotein (oxidized LDL) have been added to these factors.

Oxidized LDL has been shown to increase the expression of proinflammatory genes, causing monocyte entry into the vascular wall and vascular endothelial cell dysfunction. Oxidized LDL also takes part in the transformation of macrophages into foam cells in atherosclerotic plaque. In some pathological conditions, such as acute myocardial infarction (AMI) and coronary artery disease (CAD), elevated levels of oxidized LDL have been reported. Oxidized LDL inhibits nitric oxide production, causing endothelial dysfunction. Oxidized LDL also induces proatherogenic genes such as endothelium-leukocyte adhesion molecules and smooth muscle growth factors [6].

Lectin-like oxidized LDL receptor-1 (LOX-1) has been identified as the major receptor for oxidized LDL. This receptor is considered to be an important molecule responsible for the binding and entry of oxidized LDL into endothelial cells. In large arteries; LOX-1 has been shown to be expressed in endothelial cells, macrophages, vascular smooth muscle cells, monocytes, platelets, and fibroblasts to bind oxidized LDL [7, 8].

Increased carotid artery intima-media thickness (CIMT) is associated with many cardiovascular risk factors [9]. CIMT has been shown to reflect the distribution and the severity of atherosclerosis, correlating well with coronary artery atherosclerosis. Therefore, the measurement of CIMT is considered a very good indicator of subclinical atherosclerotic cardiovascular disease (CVD) [10].

In our study, we aimed to demonstrate whether LOX-1 and CIMT; which are the indirect biomarkers of atherosclerosis, are correlated with cardiovascular risk markers in hemodialysis patients.

METHODS

The study included 28 patients, who had undergone hemodialysis treatment for more than 6 months and 19 healthy individuals as the control group.

Patients with documented atherosclerotic cardiovascular disease, peripheral vascular disease, nicotine and alcohol use, active infection, severe liver and heart failure, and diabetes mellitus were excluded. The study protocol was approved by the Research Review Board of the Ministry of Health Bursa Yuksek Ihtisas Training and Research Hospital (Date: 01.10.2010, Decision no: 2010/2). Informed consent was obtained from all individuals participating in the study.

Medical history was obtained from all participants and general physical examinations were performed. Age, gender, smoking status, drug use, height, weight, and body mass index of the participants were noted. After the participant rested for 20 minutes; the arterial blood pressure was measured from the brachial artery with a mercury sphygmomanometer with an adult-type cuff, while the patient was in the sitting position. A systolic blood pressure of more than 140 mmHg and a diastolic pressure of more than 90 mmHg were accepted as hypertension. The participants with serum total cholesterol levels of > 200 mg/dl and/or triglyceride levels of > 150 mg/dl and/or patients taking lipid-lowering drugs were considered hyperlipidemic patients. The body weight, height, and waist circumference of the participants were measured. Body mass index (BMI) was calculated using the following formula: weight (kg)/height (m) [2].

Biochemical Analysis

Venous blood samples were collected from the participants for testing the levels of serum creatinine, urea, sodium, potassium, calcium, phosphorus, albumin, total protein, uric acid, total cholesterol, triglyceride, HDL, LDL, fasting blood sugar (FBS), parathormone, C-reactive protein (CRP), and LOX-1. Blood samples collected at least 8 hours of fasting in the morning. In hemodialysis patients, the samples were collected in the same time interval but before the hemodialysis session. To test LOX-1 levels, 5 cc blood was drawn into anticoagulant-free tubes and centrifuged for 5 minutes at 5000 rpm to separate the sera. Then, all samples were stored at -80°C by the time of the analysis of all samples together. Other laboratory analyses were performed daily. Serum LOX-1 levels were determined by using commercially available human lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) ELISA kit (Uscn Life Science Inc. Wuhan).

Carotid Artery Ultrasound Examinations

Carotid artery ultrasound examinations of the participants were performed by an experienced cardiology specialist at Bursa Yuksek Ihtisas Training and Research Hospital. The right and left carotid arteries were visualized when the participants were in the supine position and their heads were extended. Toshiba Nemio 20 high-resolution B-mode ultrasound and an 8 MHz probe were used for the measurements. The interfaces between the intima and lumen and between the media and adventitia are expressed as intimal media thickness (IMT). IMT values were calculated by taking the mean of a total of 12 measurements; which comprised two measurements at the main carotid artery level (2 cm proximal to the bulbous), two at the level of the carotid bulb, and two from the internal carotid arteries all from both sides in each participant.

Statistical Analysis

'SPSS for Windows version 13.0' software of the Department of Biostatistics of Uludag University School of Medicine was used for the statistical analyses in our study. In the study; continuous variables have been presented as median and minimum and maximum values, while categorical variables have been presented as numbers and percentages. The conformity of the continuous variables to a normal distribution was examined with the Shapiro Wilk test. Based on the results obtained from the Shapiro Wilk test, the Mann-Whitney U test was used for comparisons between the groups. Pearson's chi-square and Fisher's exact chi-square tests were used for comparing the categorical variables between the groups. Correlation analysis was performed in order to determine the associations of the variables. Pearson's and Spearman's correlation coefficients were calculated. A p - value of < 0.05 was considered statistically significant in the study.

RESULTS

A total of 47 individuals, including hemodialysis patients and the healthy control group participants, were included in the study. The hemodialysis group consisted of 28 (59.6%) individuals and the control group consisted of 19 (40.4%) individuals and. The mean age in the hemodialysis and control group was 46.5 and 40 years, respectively. When the control group and the hemodialysis group were compared, significant differences were observed in the mean systolic and diastolic blood pressure between the groups (Table 1). The hypertensive nephropathy is the most cause of disease in the hemodialysis group (Table 2).

Urea, creatinine, parathormone, phosphorus, potassium, serum triglyceride, serum CRP and uric acid levels were higher in the dialysis group compared to the control group and these elevations were statistically significant. Serum total cholesterol, LDL-cholesterol, and HDL-cholesterol levels were higher in the control group compared to the dialysis group with statistically significant differences (Table 3). The mean IMT value were significant higher in dialysis group compared with control group (0.90 mm and 0.70 mm,

Table 1. Clinical charasteristic features of patient and control group

Charasteristics	Hemodialysis (n= 28)	Control (n= 19)	*p value*
	Median (min-max.)	Median (min-max.)	
Age (years)	46.5 (22-67)	40 (33-68)	> 0.05
Gender (female/male)	10/18	16/3	> 0.05
BMI (kg/m²)	23 (16-39)	26 (20-31)	> 0.05
Dialysis duration(month)	48(12-132)	-	
SBP (mmHg)	130 (100-160)	110 (90-130)	**< 0.01**
DBP (mmHg)	80 (60-90)	70 (60-90)	**0.03**
Smoke	9	3	> 0.05

P value: for comparison of hemodialysis patients and controls (t test for continues variables and χ^2 test for categorical, as variables). The patients with hemodialysis and controls were similar according to the socioeconomic status, racial and ethnic background, and, dietary and/or physical activity habits, religion

Table 2. Causes of chronic kidney disease

	n	%
Hypertensive nephropathy	10	35.7
Glomerular disease	6	21.4
Unknown etiology	**5**	17.8
Obstructive uropathy	**5**	17.9
Chronic tubulointerstitial nephritis	2	7.2

$p < 0.001$) (see Table 3).

The mean serum LOX-1 levels of the control and dialysis groups were 0.213 ng/ml and 0.172 ng/ml, respectively. There was not a statistically significant difference in the levels of LOX-1 between the two study groups (Table 3).

DISCUSSION

The role of atherosclerosis in the aetiology of many diseases and the resulting high morbidity and mortality rates have increased the interest in this subject matter with growing importance. Prevention of atherosclerosis-associated disorders and inhibition of atherogenesis in cases with emergent diseases, or even the regression of atherogenesis, can be made possible by eliminating risk factors [11].

Carotid arteries are appropriate sites to detect the thickening; which is a good indicator of general atherosclerosis. Several studies that demonstrate the relationship between cardiovascular diseases and carotid atherosclerosis are available in the literature. High CIMT is considered an indicator of generalized ather-

Table 3. Biochemical parameters and carotis intima media thickness in study group

	Hemodialysis (n= 28)	Control (n= 19)	*p value*
	Median (min-max.)	Median (min-max.)	
Hemoglobin (g/dL)	11.35 (9.2-15.59)	12.6 (10.1-14.9)	**007**
Hematocrit (%)	33.8 (27- 45.3)	38.2 (32.2-43.9)	**< 0.001**
Leukocyte (mm³)	5950 (3200-13500)	6300 (4400-10200)	>0.05
Sodium (mEq/L)	139 (130-144)	139 (137-145)	> 0.05
Potassium (mEq/L)	4.95 (3.80-6.60)	4.40 (3.80-4.90)	**< 0.001**
Calcium (mg/dL)	8.80 (7.20-10.10)	9.10 (8.20-9.90)	0.34
Phosphorus (mg/dl)	5.45 (3.50-16)	3.67 (2.20-4.25)	**< 0.001**
Glucose (mg/dL)	88.5 (56-172)	96 (76-116)	> 0.05
Urea (mg/dL)	127 (35-190)	25 (15-36)	**< 0.001**
Creatinine (mg/dL)	9.20 (1.90-13.0)	0.70 (0.48-1.02)	**< 0.001**
Uric acid (mg/dL)	5.80 (3.70-8.80)	3.70 (2.80-6.70)	**< 0.001**
Total cholesterol (mg/dL)	147.5 (97-304)	184 (110-253)	**0.001**
Triglycerides (mg/dL)	138 (63-264)	67 (41-175)	**< 0.001**
LDL (mg/dL)	83.50 (42-225)	120 (40-182)	**0.02**
HDL (mg/dL)	38 (23-64)	54 (36-73)	**< 0.001**
Total protein (g/dL)	6.70 (5-8.10)	7.20 (6.20-7.80)	**0.013**
Albumine (gr/dl)	3.80 (2.70-4.90)	4.50 (3.80-4.90)	**< 0.001**
Parathormone (pg/mL)	237 (6-1656)	42 (10-114)	**< 0.001**
CRP (mg/L)	5.25 (1.00-34.00)	1.00 (0.10-12.70)	**< 0.001**
LOX-1 (ng/mL)	0.172 (0.12-0.54)	0.213 (0.11-0.42)	> 0.05
CIMT (mm)	0.90 (0.60-1.30)	0.70 (0.50-0.80)	**< 0.001**

P value: for comparison of patients with hemodialysis and controls (t test for continuos variables and χ^2 test for categorical, as variables).

osclerosis [10]. Several studies are available in the literature demonstrating increased CIMT in dialysis patients compared to the normal population. Kumar *et al*. [12] in 2009; in a study on 30 hemodialysis patients and a healthy control group, found significantly higher values of CIMT in hemodialysis patients compared to the healthy control group. Prasad *et al*. [13] compared CIMT of 62 diabetic and nondiabetic peritoneal dialysis patients and 62 healthy individuals in the control group and demonstrated that CIMT was higher in the patient group compared to the healthy control group. In our study, in accordance with the studies in the literature, the CIMT value in the dialysis group was found to be statistically significantly higher than that of the control group (0.90 mm vs 0.70 mm in; $p < 0.001$).

Many studies have supported that LOX-1 and atherosclerosis are closely associated. Most of the toxic effects of oxidized LDL are regulated by the LOX-1 receptor. LOX-1 expression has been shown to increase in atherosclerotic lesions in humans and experimental animal models [14].

In a study by Sakurai *et al*. [15] on endothelial dysfunction, LOX-1 was demonstrated to be closely associated with high levels of oxidative stress. Hayashida *et al*. [16] showed that; compared to the healthy control group, the serum LOX-1 level was significantly higher in acute coronary syndrome patients with symptomatic coronary heart disease. In our study, LOX-1 levels were compared between hemodialysis patients and healthy individuals. No significant differences were found in LOX-1 levels between the groups. Studies have demonstrated that both statins, angiotensin converting enzyme (ACE) inhibitors and angiotensin-2 receptor (AT-2) blockers reduce serum LOX-1 levels. Li *et al*. [17] showed that both simvastatin and atorvastatin therapy reduced the LOX-1 expression in coronary artery endothelial cells. In vitro studies have demonstrated that statins and angiotensin converting enzyme (ACE) inhibitors, inhibit oxidized LDL-induced oxidative stress, the expression of adhesion molecules, and the release of LOX-1 [18]. In our study, some of the patients in the dialysis group were receiving treatment for statin and ACE inhibitors/AT-2 blockers for hypertension and hyperlipidemia. We thought that the low levels of lox-1 in hemodialysis patients may be due to these drugs.

Determining the presence of conventional risk factors, besides uremia-specific clinical and metabolic abnormalities is highly important in chronic kidney disease-associated premature atherosclerosis. Conventional risk factors are found to increase in uremia but still inadequate alone to explain the presence of accelerated atherosclerosis. In addition to recent studies showing that atherosclerosis and inflammation are closely associated, many studies report that CRP is also closely associated with CIMT or other indicators of atherosclerosis [19-21]. This relationship is reported to exist in patients with chronic kidney failure and in patients receiving hemodialysis, too. Zoccali *et al*. [19] investigated the relationship between inflammatory processes and atherosclerosis in 138 chronic dialysis patients and concluded that CRP was found high in patients with carotid atherosclerosis (high CIMT) and that inflammation could play a role in the pathogenesis of atherosclerosis. Stenvinkel *et al*. [20] study showed that compared to the control group of healthy individuals, chronic kidney failure patients had high CIMT along with high prevalences of carotid plaques and malnutrition and that CRP was found high in malnutrition. Owen *et al*. [22] showed that serum CRP levels were significantly higher in hemodialysis patients compared to the healthy control group and that CRP as a marker of inflammation had a predictive value for cardiovascular mortality. In our study, serum CRP levels were found to be significantly higher in the hemodialysis group compared to the control group consistent with the information in the literature. Although the small number of patients causes limitations, the fact that it was more specifically performed on hemodialysis patients adds value to the study.

CONCLUSION

In conclusion, cardiovascular risk markers of CIMT and CRP were found high in the hemodialysis patient group of our study as expected. No significant differences were detected in serum LOX-1 levels; which is an important atherosclerosis marker. It will be appropriate to interpret the results of the present study by carrying out further extensive studies on this subject matter.

Authors' Contribution

Study Conception: TD, SK; Study Design: TD,

SK; Supervision: TD, SK; Funding: TD; Materials: TD, SK; Data Collection and/or Processing: TD; Statistical Analysis and/or Data Interpretation: SK; Literature Review: TD; Manuscript Preparation: TD, MGG and Critical Review: TD, MGG.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

REFERENCES

1. Hakeem A, Bhatti S, Chang SM. Screening and risk stratification of coronary artery disease in end-stage renal disease. JACC Cardiovasc Imaging 2014;7:715-28.
2. Ardhanari S, Alpert MA, Aggarwal K. Cardiovascular disease in chronic kidney disease: risk factors, pathogenesis, and prevention. Adv Perit Dial 2014;30:40-53.
3. Sarnak MJ, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. Am J Kidney Dis 2000;35 (4 suppl 1):S117-31.
4. Sarnak MJ, Coronado BE, Greene T, Wang SR, Kusek SW, Beck GJ, et al. Cardiovascular disease risk factors in chronic renal insufficiency. Clin Nephrol 2002;57:327-35.
5. Zoccali C, Mallamaci F, Tripepi G. Traditional and emerging cardiovascular risk factors in end-stage renal disease. Kidney Int Suppl 2003;85:105-10.
6. Vecchione L, Gargiu E, Borgiani P, Predazzi I, Mango R, Romeo F, et al. Genotyping OLR1 gene: a genomic biomarker for cardiovascular diseases. Recent Pat Cardiovasc Drug Discov 2007;2:147-51.
7. Moriwaki H, Kume N, Kataoka H, Murase T, Nishi E, Sawamura T, et al. Expression of lectin-like oxidized low density lipoprotein receptor-1 in human and murine macrophages: up-regulated expression by TNF-α. FEBS Lett 1998;440:29-32.
8. Aoyama T, Chen M, Fujiwara H, Masaki T, Sawamura T. LOX-1 mediates lysophosphatidylcholine induced oxidized LDL uptake in smooth muscle cells. FEBS Lett 2000;467:217-20.
9. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intimamedia thickness: a systematic review and meta-analysis. Circulation 2007;115:459-67.
10. Carpenter M, Sinclair H, Kunadian V. Carotid intima media thickness and its utility as a predictor of cardiovascular disease: a review of evidence. Cardiol Rev 2016;24:70-5.
11. Jungers P, Massy ZA, Nguyen Khoa T, Fumeron C, Labrunie M, Lacour B, et al. Incidence and risk factors of atherosclerotic cardiovascular accidents in predialysis chronic renal failure patients: a prospective study. Nephrol Dial Transplant 1997;12:2597-602.
12. Kumar, KS, Lakshmi HY, Rao PVS, Das GC, Kumar VS. Carotid intima-media thickness in patients with end-stage renal disease. Indian J Nephrol 2009;19:13-4.
13. Prasad N, Kumar S, Singh A, Sinha A, Chawla K, Gupta A, et al. Carotid intimal thickness and flow-mediated dilatation in diabetic and nondiabetic continuous ambulatory peritoneal dialysis patients. Perit Dial Int 2009;29 Suppl 2:S96-101.
14. Puccetti L, Pasqui AL, Bruni F, Pastorelli M, Ciani F, Palazzuoli A, et al. Lectin like oxidized-LDL receptor-1 (LOX-1) polymorphisms influence cardiovascular events rate during statin treatment. Int J Cardiol 2007;25:41-7.
15. Sakurai K, Sawamura T. Stress and vascular responses: endothelial dysfunction via lectin-like oxidized low-density lipoprotein receptor-1: close relationships with oxidative stress. J Pharmacol Sci 2003;91:182-6.
16. Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, Inada T, et al. Serum soluble lectin like oxidized-LDL receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. Circulation 2005;112:812-8.
17. Li D, Chen H, Romeo F, Sawamura T, Saldeen T, Mehta JL. Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: role of LOX-1. J Pharmacol Exp Ther 2002;302:601-5.
18. Sugiyama M, Ohashi M, Takase H, Sato K, Ueda R, Dohi Y. Effects of atorvastatin on inflammation and oxidative stress. Heart Vessels 2005;20:133-6.
19. Zoccali C, Benedetto FA, Mallamaci F, Tripepi G, Fermo I, Foca A, et al. Inflammation is associated with carotid atherosclerosis in dialysis patients. Creed Investigators. Cardiovascular Risk Extended Evaluation in Dialysis Patients. J Hypertens. 2000;18:1207-13.
20. Stenvinkel P, Heimburger O, Paultre F, Diczfalusy U, Wang T, Berglund, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int 1999;55:1899-911.
21. Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Lancet 1997;349:462-6.
22. Owen WF, Lowrie EG. C-reactive protein as an outcome predictor for maintanence hemodialysis patients. Kidney Int 1998;54:627-36.