



# A Case-Control Study Investigating Cardiovascular Health in Maintenance Hemodialysis Patients through Oxidative Stress Biomarkers and Carotid Artery Intima-Media Thickness

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## Abstract

**Introduction:** Chronic kidney disease (CKD) is a major risk factor for the development of cardiovascular disease (CVD), and it is the leading cause of morbidity and mortality in end-stage renal disease (ESRD) patients receiving maintenance hemodialysis (MHD).

**Aim:** This study aims to evaluate biomarkers of oxidative stress (OS) and carotid artery intima-media thickness as predictors of cardiovascular health among MHD patients.

**Materials and methods:** We divided 135 participants in this prospective case-control study into three groups: group A included 45 healthy controls, group B included 45 ESRD patients receiving MHD for less than three years, and group C included 45 ESRD patients receiving MHD for more than three years. Participants aged 18–50 years, not taking antioxidant supplements, and willing to participate were included, excluding those with chronic illnesses, prior cardiac disease, or acute renal failure. Data collected included demographics, MHD duration, medical history, lipid profile, common carotid artery intima-media thickness (CCA-IMT), and some biochemical parameters such as oxidized LDL (Ox-LDL), malondialdehyde (MDA), and superoxide dismutase (SOD).

**Results:** This study included 135 participants divided into three groups (A, B, and C) based on the MHD duration. Significant differences were observed in OS markers and lipid profiles across the groups ( $p < 0.001$ ). Group C exhibited the highest levels of Ox-LDL and MDA, indicating increased OS, and the lowest SOD levels compared to groups A and B. Positive correlations were found between Ox-LDL and LDL-cholesterol (LDL-C) levels, with the strongest correlation in group C ( $r = 0.684$ ,  $p < 0.05$ ). CCA-IMT progressively increased from group A to group C, with significant differences in right, left, and mean CCA-IMT ( $p < 0.001$ ). Multivariate analysis revealed a positive association between Ox-LDL levels and CCA-IMT ( $p < 0.01$ ).

**Conclusion:** Increased OS, evident by elevated Ox-LDL and reduced antioxidant levels, is linked to unfavorable lipid profiles and carotid atherosclerosis progression in MHD patients. Prolonged MHD duration contributes to heightened OS and increased atherosclerosis development. Ox-LDL emerges as a predictor of CVD risk in this population.

## Keywords

atherosclerosis, carotid artery intima-media thickness, maintenance hemodialysis, oxidative stress

## INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem across the world, with maintenance hemodialysis (MHD) being the widely used renal replacement therapy (RRT) for end-stage renal disease (ESRD) patients.<sup>[1,2]</sup> It affects approximately 13.4% of the population globally and the data from the United States suggest an adult prevalence of 13.1%, which has risen over time.<sup>[3]</sup> Due to the scarcity of data, the precise burden of CKD is still unknown in Pakistan, as there is no national renal registry in Pakistan and hence there is no credible estimate of CKD and ESRD prevalence.<sup>[1]</sup> However, according to a review by Imtiaz et al., the overall prevalence of CKD in Pakistan is 16.7%.<sup>[4]</sup>

CKD is a key risk factor for developing cardiovascular diseases (CVD) and the leading cause of morbidity and mortality.<sup>[3,5]</sup> Even after making adequate modifications, CVD mortality is 10 to 20 times greater in ESRD patients than in the general population.<sup>[6]</sup> According to the European Registry, patients on RRT have a 35%–50% higher risk of CVD, with incidences of coronary heart disease and ventricular hypertrophy at around 40% and 70%, respectively.<sup>[2,6]</sup> Identification of CVD risk variables is critical for evaluating therapy impact and lowering the rate of mortality. Traditional risk factors (e.g., hypertension, hyperlipidemia, diabetes, physical inactivity, smoking, and advanced age) are exacerbated by nontraditional risk factors associated with renal failure.<sup>[6–8]</sup> Non-traditional risk factors, notably oxidative stress (OS) and inflammation, are important in the progression of CVD. These causes are tightly interwoven and mutually reinforcing, resulting in CVD in MHD patients.<sup>[9]</sup> MHD is also linked to exacerbating OS, which contributes to inflammation, decreased endothelial function, and increased risk of atherosclerosis.<sup>[8]</sup> It is essential to identify useful indicators predicting the onset of CVD in CKD patients and to recognize characteristics related to various OS indicators to design tailored therapeutics aimed at minimizing OS.<sup>[9,10]</sup>

The change in the equilibrium between pro-oxidants and antioxidants results in OS. Hence, this imbalance leads to the formation of reactive oxygen species (ROS) and free radicals, both of which are harmful to body cells. The dialysis procedure itself causes a reduction in antioxidants and an increase in oxidative molecules, exacerbating the already established OS. Uremic condition, in combination with dialysis, is a substantial risk factor for OS-related complications.<sup>[2]</sup> Although superoxide dismutase (SOD) plays a vital role in combating ROS, a decrease in total antioxidants has been reported in CKD patients undergoing MHD. Oxidized low-density lipoprotein (Ox-LDL) and

serum malondialdehyde (MDA) have been linked to CVD and OS in ESRD patients.<sup>[11]</sup> Because of its mutagenic and cytotoxic properties, MDA has a potential role in CVD development and is a commonly utilized indicator for OS yield through the oxidative degeneration of lipids and a single strong predictor of widespread CVD illness, and it is also implicated in the pathogenesis of atherosclerosis in the literature.<sup>[2,9]</sup> Ox-LDL levels correlate with left ventricular hypertrophy in juvenile MHD patients and peripheral artery disease severity. Multiple studies have reported an increase in Ox-LDL levels following a dialysis session.<sup>[11]</sup> LDL metabolism in CKD has been widely researched, often revealing elevated LDL-C levels in early CKD stages. Interestingly, ESRD patients might present with normal or decreased LDL-C and total cholesterol levels, unveiling a negative correlation between cholesterol levels and mortality in this cohort. Nevertheless, it has been observed that Ox-LDL levels are higher than LDL-C levels in ESRD patients, indicating a complex interaction between traditional lipid markers and OS in this population.<sup>[12,13]</sup> It is interesting to mention that the conventional lipid profile, routinely performed on MHD patients, does not provide information on lipid peroxidation levels. Ox-LDL arises from the biochemical modification of native LDL by free radicals, leading to alterations in its structure and properties.<sup>[14]</sup> These changes result in Ox-LDL being smaller and more capable of penetrating the intima layer of blood vessels, initiating an inflammatory response that can lead to plaque formation and the progression of atherosclerosis.<sup>[14,15]</sup> Atherosclerosis is commonly recognized as a pathogenic process involved in the progression of ischemic heart diseases.<sup>[15]</sup> According to the literature, atherosclerosis begins in the initial stages of CKD.<sup>[12]</sup> The American Heart Association recommends carotid artery ultrasonography, a noninvasive imaging technique, to assess the risks associated with cerebrovascular and heart diseases.<sup>[11]</sup> In MHD patients, measuring Ox-LDL cholesterol levels enhances atherosclerotic CVD prediction over total serum LDL-C levels. Additionally, MDA levels offer potential as an early diagnostic for atherosclerosis.<sup>[11,16]</sup>

Ultrasonography is widely used to diagnose advanced atherosclerosis by assessing carotid intima-media thickness.<sup>[11,15,17]</sup> However, it does not detect the early biochemical processes preceding plaque formation. Our study aims to fill this gap by identifying a biomarker signaling these processes before structural changes visible via ultrasonography. Early detection is crucial for timely intervention, especially in Pakistan, where CVDs are a leading cause of mortality among MHD patients.<sup>[1,2,18]</sup> By establishing Ox-LDL as an early indicator of OS and lipid peroxidation, we

are not only enhancing our ability to detect the early stages of atherosclerosis but also providing a foundation for preventive strategies aimed at reducing cardiovascular risk in this vulnerable population. CKD impacts social life and productivity. Despite the global progress in dialysis, there is insufficient literature examining the relationship between MHD and the risk of CVD in developing countries, particularly among the population in Pakistan. The suboptimal survival outcomes in Pakistan can be attributed to the ineffective delivery of hemodialysis care, which falls behind international standards.<sup>[1]</sup> Limited local data on chronic hemodialysis hampers understanding of long-term survival and outcome factors.

## AIM

Our study explores the link between OS and CVD in MHD patients, aiming to illuminate early diagnosis and innovative treatment approaches tailored to this high-risk group.

## MATERIALS AND METHODS

This was a prospective case-control study carried out at the Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi, in collaboration with the Departments of Radiology and Nephrology. The sample size was determined using OpenEpi, following the available research literature.<sup>[19]</sup> The reporting of this case-control study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

### Participants

The subjects were categorized into three groups. Group A, referred to as the control group, comprised forty-five healthy participants recruited from the hospital. Group B consisted of forty-five individuals who had undergone MHD for up to three years, while group C included forty-five participants with MHD duration exceeding three years. Participants of both sexes, aged between eighteen and fifty years, who were not using additional antioxidants, had undergone MHD as RRT, and expressed willingness to take part were included. Conversely, individuals with significant chronic illnesses, such as tuberculosis or any malignancy, a history of prior cardiac disease, and those undergoing hemodialysis for acute renal failure, were excluded from the study.

### Data collection tool

A detailed questionnaire was used for data collection, including demographic information, anthropometric measurements, duration of MHD, comprehensive medical and medication history, and a general physical examination. Vital signs such as systolic blood pressure (SBP) and di-

astolic blood pressure (DBP), pulse pressure, respiratory rate, and temperature were recorded. The assessment included biochemical parameters like total cholesterol (TC), triglycerides (TGs), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), serum oxidized LDL (Ox-LDL), serum malondialdehyde (MDA), and plasma superoxide dismutase (SOD) levels. Ultrasonographic measurements of right and left common carotid artery intima-media thickness (CCA-IMT) were also obtained.

## Laboratory parameters

On non-dialysis days, all biochemical analyses were conducted after an overnight fast. A sterile disposable syringe was utilized to draw a 5-ml venous blood sample, which was subsequently transferred into vacutainers.

For quantifying TC concentration in the blood, the enzymatic colorimetric technique (Kit Cat. No. CH 8240) manufactured by Merck, France, was employed, and the results were expressed in milligrams per deciliter (mg/dL). Serum HDL-C levels were measured using an enzymatic kit method, also reported in milligrams per deciliter (mg/dL). Similarly, the glycerol-3-phosphate oxidase phenol amino phenazone method determined TGs levels in the serum in milligrams per deciliter (mg/dL).

To calculate LDL-C, the Friedewald formula was applied:  $LDL-C = TC - (HDL-C + TGs/5)$ . Serum Ox-LDL levels were assessed in U/l using the Human ELISA Kit for Ox-LDL, Catalogue Number CEA527Hu. The marker of OS, MDA, in the blood was measured according to the Okhawa et al. technique, with results expressed as thiobarbituric acid-reacting substances in nmol/ml. Additionally, the antioxidant enzyme SOD activity in plasma was determined as U/L using the Kono technique. The rationale for selecting Ox-LDL, MDA, and SOD as biomarkers is grounded in their well-established involvement in OS and associated biochemical pathways. By established scientific knowledge, Ox-LDL stands out as a more specific biomarker for atherosclerosis owing to its smaller size, facilitating its easy passage through the intima-media layer of blood vessels. Additionally, SOD represents the largest endogenous antioxidant in the body, and MDA serves as a relatively stable ROS, rendering its levels more reliable for predictive purposes. These chosen biomarkers function as dependable indicators of OS damage, lipid peroxidation, and antioxidant defense mechanisms. CCA-IMT was measured via ultrasonography to evaluate atherosclerosis. The thickness of the tunica media and tunica intima of the common carotid artery was expressed in millimeters (mm) using high-resolution B-mode ultrasonography.

The predictors studied are common CVD risk factors like high cholesterol, age, sex, and MHD duration. Potential confounding variables that could affect the relationship between Ox-LDL levels and cardiovascular outcomes in ESRD patients include age, sex, lifestyle factors (diet, exercise, smoking), and dialysis-related factors (frequency

and duration of dialysis sessions) that may impact oxidative stress levels and inflammation. Effect modifiers that could change the effect of Ox-LDL on cardiovascular outcomes in MHD patients include genetic predispositions to oxidative stress and atherosclerosis, the effectiveness of anti-inflammatory or cholesterol-lowering treatments, and the presence of other health conditions. We used consecutive sampling to include all eligible participants during the study period. A broad age range and inclusion of both sexes aimed to improve generalizability. Excluding those taking antioxidant supplements helped isolate the effects of oxidative stress related to ESRD and its treatment. Participants with serious chronic illnesses, history of CVD or acute renal failure were excluded to minimize confounding effects on the outcomes of interest. Detailed data on demographics, duration of MHD and medical history were collected to control potential confounders in the analysis. This allows for a better understanding of how factors impact the relationship between oxidative stress markers and cardiovascular outcomes in ESRD patients.

### Outcomes

The primary outcome of interest in this study is the development of CVD, including atherosclerosis, among patients with ESRD. Secondary outcomes include the progression of atherosclerosis, measured by changes in carotid intima-media thickness over time.

### Data analysis procedure

Data was stored and analyzed utilizing IBM-SPSS version 23.0. The data encompassing profiles, serum parameters, OS markers, lipid profiles, and intima-media thickness underwent normality testing through the Shapiro-Wilk test. For parameters not following a normal distribution, the median with interquartile range was reported. Comparison across the three groups was conducted using the Kruskal-Wallis test, and the duration of MHD between the two groups was assessed through the Mann-Whitney U test. Post-hoc analysis of significant parameters was performed

using Tukey’s HSD test. Non-parametric Spearman Rank Correlation was employed to investigate the correlation between Ox-LDL, OS, and lipid profile. Multivariate regression models were used to explore the associations between the studied parameters. We adjusted for potential confounders including age, sex, BMI, SBP, DBP, MDA, SOD, TGs, TC, HDL-C, and LDL-C levels. Interaction terms were included where appropriate to assess potential effect modification. Statistical significance was set at  $p < 0.05$ .

### Ethical approval and consent to participate

The study protocol adhered to the ethical guidelines set by both institutional and national research committees, as well as the principles outlined in the Helsinki Declaration. Ethical authorization was granted by Jinnah Postgraduate Medical Center’s institutional review committee. Written, understood and voluntary consent was taken from the participants. Data was coded for confidentiality and accessible only to the principal investigator.

## RESULTS

The study included a total of 135 participants, equally divided into three groups (45 participants each). Groups A and C comprised 27 (60%) men and 18 (40%) women, while group B had 28 (63%) men and 17 (37%) women. The median age of participants was 33.5 years in group A, 35.5 years in group B, and 43.5 years in group C.

**Table 1** presents the median comparison of participant profiles across the study groups. Significant differences were observed in age, BMI, SBP and DBP using the Kruskal-Wallis test ( $p < 0.001$  for age, SBP, and DBP;  $p = 0.006$  for BMI). Post-hoc analysis revealed that group C had a significantly higher median age compared to groups A and B, while median BMI was significantly lower in group C than in group B. The median SBP and DBP differed significantly among all three groups. The median duration of MHD was 2 years for group B and 8 years for group C, with a signifi-

**Table 1.** Comparison of demographic and other baseline parameters across study groups

Parameters	Group A	Group B	Group C	P-value	Significant pairwise comparison
	Median (IQR)	Median (IQR)	Median (IQR)		
Age (years)	33.5 (12)	35.5 (13)	43.5 (6)	<0.001*	(2, 3)
BMI (kg/m <sup>2</sup> )	23.21 (3.27)	25.46 (6.9)	21.6 (5.67)	0.006*	3
SBP (mmHg)	110 (10)	130 (10)	160 (20)	<0.001*	(1, 2, 3)
DBP (mmHg)	70 (70-60)	90 (90-80)	90 (100-90)	<0.001*	(1, 2, 3)
Weight (kg)	66.5 (9)	69 (6)	64 (11)	0.07	-
Height (m)	1.68 (0.22)	1.58 (0.22)	1.55 (0.22)	0.06	-
Duration of dialysis (years)	-	2 (1)	8 (3)	<0.001*	-

1: Difference between A and B; 2: Difference between A and C; 3: Difference between B and C

cant difference between the two groups ( $p<0.001$ ).

The analysis revealed significant differences in oxidative stress and lipid profile parameters across the three groups ( $p<0.001$ ) and significant pairwise differences were observed for most parameters among the three groups as shown in **Table 2**. Group C exhibited the highest median levels of Ox-LDL at 74 U/L and MDA at 29.9 nmol/ml, along with the lowest SOD levels of 46.2 U/L, indicating increased OS compared to groups A and B. TGs levels were also the highest in group C at 186.5 mg/dl. While TC was the highest in group B at 148.5 mg/dl, HDL-C levels were lowest in group C at 20 mg/dl. LDL-C levels were comparable between groups A and C but lower than group B.

**Table 3** presents significant correlations between Ox-LDL, MDA, SOD and lipid profile parameters across the three groups, using Spearman's rank correlation within each study group. In group A, Ox-LDL showed a significant positive correlation with LDL-C levels ( $r=0.505$ ,  $p<0.05$ ). In group B, MDA had a significant positive correlation with HDL-C levels ( $r=0.388$ ,  $p<0.05$ ), while Ox-LDL was positively correlated with LDL-C levels ( $r=0.386$ ,  $p<0.05$ ). In group C, Ox-LDL exhibited a strong positive correlation with LDL-C ( $r=0.684$ ,  $p<0.05$ ) and a moderate positive correlation with MDA ( $r=0.356$ ). Additionally, SOD showed a significant negative correlation with LDL-C ( $r=-0.601$ ,  $p<0.05$ ) and HDL-C ( $r=-0.584$ ,  $p<0.05$ ) in this group. The

**Table 2.** Comparison of oxidative stress and lipid profile parameters across study groups

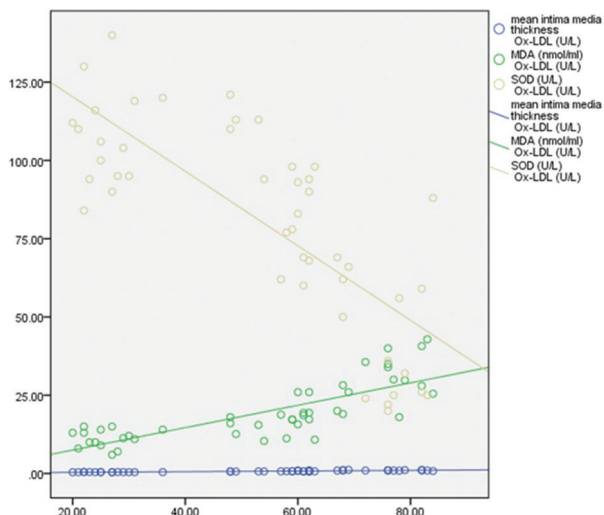
Parameters	Group A	Group B	Group C	P-value	Significant pairwise comparison
	Median (IQR)	Median (IQR)	Median (IQR)		
Ox-LDL (U/L)	24.5 (7.75)	59 (4.5)	74 (9.75)	<0.001*	(1, 2, 3)
MDA (nmol/ml)	11 (3.87)	16.85 (5.4)	29.9 (11.62)	<0.001*	(1, 2, 3)
SOD (U/L)	108.53 (24.75)	85.23 (17.5)	46.2 (32.25)	<0.001*	(1, 2, 3)
TGs (mg/dl)	86 (29.75)	123 (37.25)	186.5 (32)	<0.001*	(1, 2, 3)
TC (mg/dl)	100.5 (30.75)	148.5 (42)	128 (49.5)	<0.001*	(1, 2)
HDL-C (mg/dl)	43 (8)	31 (6)	20 (7)	<0.001*	(1, 2, 3)
LDL-C (mg/dl)	75.5 (12.25)	89.5 (19.75)	70.5 (32.75)	<0.001*	(1, 3)

1: Difference between A and B; 2: Difference between A and C; 3: Difference between B and C

**Table 3.** Correlations between oxidative stress markers and lipid profiles across study groups

Parameters	Group	Ox-LDL U/L	MDA nmol/ml	SOD U/L	TGs mg/dl	TC mg/dl	HDL-C mg/dl
MDA (nmol/ml)	A	0.18					
	B	0.2					
	C	0.34					
SOD (U/L)	A	0.09	0.08				
	B	-0.18	-0.364*				
	C	-0.395*	-0.722*				
TGs (mg/dl)	A	0.05	0.23	-0.1			
	B	-0.07	-0.2	0.12			
	C	0.06	0.28	-0.2			
TC (mg/dl)	A	0.09	0.24	0.1	-0		
	B	-0.27	-0.1	0.24	0.4		
	C	0.28	0.22	-0.1	-0		
HDL-C (mg/dl)	A	0.36	0.14	0.13	-0	-0	
	B	-0.16	0.388*	0.04	0	-0	
	C	-0.24	-0	-0	-0.2	-0.584*	
LDL-C (mg/dl)	A	0.505*	0.22	-0	0.1	0.416*	0.2
	B	0.386*	0.23	-0.3	0	-0	0.1
	C	0.684*	0.36	-0.601*	-0.1	0	-0

\*Correlation was considered statistically significant at  $p<0.05$  (Spearman Rank Test)



**Figure 1.** Scatter plot representing the correlation of Ox-LDL, MDA, SOD, and carotid artery intima-media thickness.

findings indicate a 39.5% negative correlation between Ox-LDL and SOD in group C, a 50.5% positive correlation between Ox-LDL and LDL-C in group A, a 38.6% positive correlation between Ox-LDL and LDL-C in group B, and a 68.4% positive correlation between Ox-LDL and LDL-C in group C. Furthermore, MDA exhibits a 36.4% negative correlation with SOD in group B and a 72.2% negative correlation with SOD in group C, respectively, as well as a 38.8% positive correlation with HDL-C in group B. Additionally, SOD demonstrates a 60.1% negative correlation with

LDL-C in group C samples, while TC shows a 58.4% negative correlation with HDL-C in group C. **Fig. 1** illustrates the overall positive and negative correlations among serum parameters, including Ox-LDL, MDA, SOD, and carotid artery IMT, using a scatter plot across all study groups.

CCA-IMT revealed significant differences across the study groups ( $p < 0.001$ ), as shown in **Table 4**. The Kruskal-Wallis test showed that the median right CCA-IMT, left CCA-IMT, and mean IMT differed significantly among groups A, B, and C. In pairwise comparisons using Tukey's HSD test, all group pairs exhibited significant mean differences in these parameters, with an increasing trend observed from group A to group C ( $p < 0.05$ ). Specifically, the median right CCA-IMT ranged from 0.44 mm in group A to 0.69 mm in group B, and 0.92 mm in group C. The median left CCA-IMT increased from 0.41 mm in group A to 0.69 mm in group B and 1.1 mm in group C.

**Table 5** presents significant findings from a multivariate analysis, showing a positive association between Ox-LDL levels and intima-media thickness (IMT), with a significant value ( $p < 0.01$ ). SBP, LDL-C levels, and MHD duration were significant predictors of Ox-LDL levels. Specifically, Ox-LDL levels were higher by 55.7 units in group B and 34.8 units in group C (both  $p < 0.01$ ). In the multivariate model, SBP, LDL-C levels, and MHD status emerged as significant predictors, while the interaction between MHD status and sex was found to be insignificant. Right CCA-IMT increased by 0.5 mm in group B and 0.3 mm in group C (both  $p < 0.01$ ), while left CCA-IMT increased by 0.7 mm in group B and 0.3 mm in group C (both  $p < 0.01$ ). Mean IMT also

**Table 4.** Comparison of carotid artery intima-media thickness (IMT) across study groups

Parameters	Group A	Group B	Group C	P-value	Significant pairwise comparison
	Median (IQR)	Median (IQR)	Median (IQR)		
Right CCA-IMT (mm)	0.44 (0.07)	0.6 (0.10)	0.92 (0.06)	<0.001*	(1, 2, 3)
Left CCA-IMT (mm)	0.41 (0.01)	0.69 (0.02)	1.1 (1.21)	<0.001*	(1, 2, 3)
Mean IMT	0.43 (0.03)	0.67 (0.07)	0.98 (1.07)	<0.001*	(1, 2, 3)

1: Difference between A and B; 2: Difference between A and C; 3: Difference between B and C.

**Table 5.** Multivariate analysis of predictors for Ox-LDL levels and carotid artery IMT

Dependent variable	Cases	Beta coefficient	Standard error	t-value	p-value	95% Confidence interval	
						Lower bound	Upper bound
Ox-LDL (U/L)	Group B	55.7	8	7	<0.01*	39.3	72
	Group C	34.8	5	7	<0.01*	24.6	44.9
Right CCA-IMT (mm)	Group B	0.5	0.1	4.9	<0.01*	0.3	0.7
	Group C	0.3	0.1	4	<0.01*	0.1	0.4
Left CCA-IMT (mm)	Group B	0.7	0.1	5.1	<0.01*	0.4	1
	Group C	0.3	0.1	3.5	<0.01*	0.1	0.5
Mean IMT	Group B	0.6	0.1	6.8	<0.01*	0.4	0.8
	Group C	0.3	0.1	5	<0.01*	0.2	0.4

\*Beta coefficient with  $p < 0.05$  was considered statistically significant (The model was adjusted for age, BMI, SBP, DBP, MDA, SOD, TC, TGs, HDL-C, and LDL-C)

increased significantly: 0.6 mm for group B and 0.3 mm for group C (both  $p < 0.01$ ). These results highlight the significant impact of MHD on Ox-LDL levels and CCA-IMT.

## DISCUSSION

The primary cause of death in individuals undergoing MHD is CVD. The main contributor to dialysis-related CVD in these patients is OS. This study aimed to investigate the relation between atherosclerotic changes in patients on MHD and markers of OS. In the present study, patients with longer durations of MHD exhibited significantly reduced BMI and weight. These findings align with the conclusions drawn by Rysz et al.<sup>[20]</sup> The decline in BMI could potentially be attributed to protein-energy wasting and dietary restrictions in individuals undergoing MHD.

We discovered that the SBP and DBP of the MHD patients were significantly higher than that of group A with a highly significant  $p$ -value  $< 0.001$ . This rise in blood pressure may be caused by fluid overload, excessive renin-angiotensin system activity, the injection of erythropoietin, or increased sympathetic nervous system activation. Our findings are in line with those of Wang et al., who found that MHD patients had a mean SBP of  $143.2 \pm 32.7$  mmHg and a mean DBP of  $79.0 \pm 15.9$  mmHg.<sup>[21]</sup> Compared to the control (group A), individuals undergoing MHD exhibited elevated blood levels of Ox-LDL, with significantly higher values observed among participants in group C. These differences were found to be statistically significant across all groups, as indicated by the  $p$ -values  $< 0.001$ . According to Manabe et al., the mean Ox-LDL concentration in MHD patients was  $74.6 \pm 28.1$  U/L.<sup>[22]</sup> Hou et al. showed that individuals receiving MHD had higher Ox-LDL levels ( $89.15 \pm 12.3$  U/L). This rise in levels is brought on by increased OS during several hemodialysis cycles, which in turn causes enhanced lipid peroxidation.<sup>[23]</sup>

We observed significant findings in Doppler ultrasonography. The mean intima-media thickness and the thickness of the right and left CCA differed, demonstrating a  $p$ -value of less than 0.001. Our findings align with those reported by Mahmoud et al. who observed increased carotid artery IMT ( $1.0 \pm 0.7$ ) in MHD patients.<sup>[24]</sup> Elevated IMT serves as a marker for the progression of atherosclerotic alterations. This outcome suggests that individuals undergoing dialysis face an increased susceptibility to developing CVD.<sup>[2]</sup> Based on these findings, it is evident that CKD patients on MHD undergo OS, leading to the generation of inflammatory mediators and Ox-LDL, both contributing to the development of atherosclerotic vascular disease. Several previous studies have investigated the carotid artery IMT ratio as a noninvasive biomarker and predictor of atherosclerotic heart disease.<sup>[20]</sup> The high levels of Ox-LDL and CCA-IMT indicate an enhanced status of lipid peroxidation in these patients.<sup>[2]</sup>

The dialysis procedure induces a decline in antioxidants and a rise in oxidative molecules, thereby exacerbating the

pre-existing OS. The uremic state, coupled with the dialysis procedure, constitutes a significant risk factor for complications. Our study reveals that, in comparison to controls, MHD patients exhibited lower SOD levels. A statistically significant increase in serum MDA was observed when cases were compared to controls showing a  $p$ -value  $< 0.001$ , consistent with previous research findings.<sup>[23]</sup> Notably, SOD levels in group C were remarkably low. Factors such as age, creatinine clearance, uremic status, the duration of MHD, the selective permeability of the dialysis machine membrane to antioxidants, and bacterial contaminants in the dialysate contribute to the potential mechanisms underlying this low level.

OS remains a prevalent issue for individuals undergoing dialysis. Our study also identified a positive correlation between Ox-LDL and MDA, along with a negative correlation with SOD. This imbalance between oxidant and antioxidant levels contributes to the occurrence of OS related complications in these patients, emphasizing the importance of monitoring these biomarkers in ESRD patients on MHD. Incorporating appropriate antioxidants into treatment regimens after assessing serum MDA levels is recommended.<sup>[2]</sup> The heightened state of OS is primarily attributed to an exogenous antioxidant-deficient diet, the accumulation of oxidative products, and the loss of antioxidant molecules during each hemodialysis session.<sup>[22]</sup> This condition is closely linked to the onset of hypertension, prolonged inflammation, and CVD. While administering antioxidants to patients undergoing MHD appears promising for preventing OS, it has not yet been integrated into routine clinical practice.<sup>[20]</sup> The potential protective impact of antioxidant therapy against cellular stress, promising to improve the cardiovascular risk profile in CKD and ESRD, needs clarification through large, prospective trials. Furthermore, routine monitoring of OS indicators in these patients is crucial to prevent the development of complications. The administration of antioxidants and statins to patients necessitates a personalized approach based on individual needs, determined through the analysis of laboratory reports encompassing biomarker levels.<sup>[2]</sup> The variability of biomarker levels across patients underscores the importance of tailored interventions. In this situation, adding Ox-LDL, MDA, and SOD tests to the usual biomarkers is recommended to improve prediction abilities. By assessing these biomarker levels, adjustments to dietary and medicinal requirements can be made, aiding physicians in formulating long-term prognoses for patients. Specifically, patients exhibiting elevated levels of Ox-LDL and increased carotid artery IMT should receive targeted counseling, designating them as high-risk individuals for vascular disorders and necessitating corresponding counseling strategies.

## CONCLUSION

Ox-LDL functions as a biomarker indicating lipid peroxidation and OS. MHD patients experience heightened OS,

making Ox-LDL a reliable indicator for detecting atherosclerotic disease in this group. SOD levels are diminished in MHD patients, while MDA levels are substantially higher in patients on MHD. OS increases as the duration of dialysis increases, and both SBP and DBP were significantly higher than those of the control group. Changes in serum Ox-LDL levels and CCA-IMT are notably apparent in hemodialysis-treated ESRD patients. Our findings reveal a significant elevation of Ox-LDL in MHD patients, serving as a marker for detecting subclinical atherosclerosis.

## Limitations

One important limitation is that our study was conducted at only one center, so our findings may not apply to a wider group of people. Unfortunately, we could not include measurements of myeloperoxidase and C-reactive protein levels due to limited resources. Also, we did not examine specific cardiac biomarkers or coronary arteries in high-risk individuals. To make our conclusions more dependable and relevant, we suggest more studies in different centers or with different groups of people. If our findings are replicated in various settings, it will make our results more trustworthy and help us understand the topic better.

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The authors have declared that no competing

## Author contributions

S.R.: conceptualization, investigation, methodology, data curation, supervision, writing – original draft, validation, visualization, project administration, writing – review & editing, resources and funding acquisition; S.K.: conceptualization, supervision, resources, methodology, visualization, investigation and validation; M.R.S.: investigation, methodology, formal analysis, data curation, software, re-

sources, funding acquisition, writing – original draft and writing – review and editing; S.S.: investigation, data curation; M.T.K.: investigation, data curation; S.B.: data curation and writing – original draft.

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# Исследование случай-контроль, изучающее состояние сердечно-сосудистой системы у пациентов, находящихся на поддерживающем гемодиализе, с помощью биомаркеров окислительного стресса и толщины интима-медиа сонной артерии

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## Резюме

**Введение и цели:** Хроническая болезнь почек (ХБП) является основным фактором риска развития сердечно-сосудистых заболеваний (ССЗ), а также основной причиной заболеваемости и смертности у пациентов с терминальной стадией почечной недостаточности (ESRD), получающих поддерживающий гемодиализ (ПГД). Целью данного исследования является оценка биомаркеров окислительного стресса (ОС) и толщины интима-медиа сонной артерии как предикторов сердечно-сосудистого здоровья у пациентов с ХБП.

**Материалы и методы:** Мы разделили 135 участников этого проспективного исследования случай-контроль на три группы: группа А включала 45 здоровых лиц контрольной группы, группа В включала 45 пациентов с ESRD, получающих гемодиализ менее трёх лет, и группа С включала 45 пациентов с ESRD, получающих гемодиализ более трёх лет. В исследование были включены участники в возрасте 18–50 лет, не принимающие антиоксидантные добавки и желающие участвовать, за исключением лиц с хроническими заболеваниями, предшествующими сердечными заболеваниями или острой почечной недостаточностью. Собранные данные включали демографические данные, продолжительность гемодиализа, историю болезни, липидный профиль, толщину интима-медиа общей сонной артерии (ССА-ИМТ) и некоторые биохимические параметры, такие как окисленный LDL (Ох-LDL), малоновый диальдегид (MDA) и супероксиддисмутаза (SOD).

**Результаты:** В это исследование было включено 135 участников, разделённых на три группы (А, В и С) в зависимости от продолжительности ПГД. Значительные различия наблюдались в маркерах ОС и липидных профилях между группами ( $p < 0,001$ ). Группа С показала самые высокие уровни Ох-LDL и MDA, что указывает на повышенную ОС, и самые низкие уровни SOD по сравнению с группами А и В. Положительные корреляции были обнаружены между уровнями Ох-LDL и LDL-холестерина (LDL-C), с самой сильной корреляцией в группе С ( $r = 0,684, p < 0,05$ ). ССА-ИМТ постепенно увеличивался от группы А к группе С, со значительными различиями в правом, левом и среднем ССА-ИМТ ( $p < 0,001$ ). Многофакторный анализ выявил положительную связь между уровнями Ох-LDL и ССА-ИМТ ( $p < 0,01$ ).

**Заключение:** Повышенная ОС, выраженная повышенным уровнем Ох-LDL и сниженным уровнем антиоксидантов, связана с неблагоприятными липидными профилями и прогрессированием атеросклероза сонных артерий у пациентов с ПГД. Длительная продолжительность ПГД способствует повышению ОС и усилению развития атеросклероза. Ох-LDL выступает в качестве предиктора риска сердечно-сосудистых заболеваний в этой популяции.

## Ключевые слова

атеросклероз, толщина интима-медиа сонной артерии, поддерживающий гемодиализ, оксидантный стресс