ORIGINAL ARTICLE

Association of Elevated Oxidized Low Density Lipoprotein to Low Density Lipoprotein Ratio (Ox LDL/LDL) with Sub Clinical Atherosclerosis in Patients Receiving Maintenance Haemodialysis

Sadia Rehman[1], Santosh Kumar[2], Kashif Nisar[3], Zara Sami[4], Fatima Mehboob[1], and Suniya Rehman[5]

ABSTRACT

Objective: To assess the oxidized low density lipoprotein (ox-LDL) to low density lipoprotein (LDL) ratio as an indicator of subclinical atherosclerosis in haemodialysis patients

Methodology: This case control study was carried out at Jinnah Post Graduate Medical Center Karachi, from January to December 2018. The haemodialysis patients were selected from the dialysis center (group B) whereas the controls were recruited from normal healthy population (group A). To access the status of atherosclerosis, doppler ultrasonography technique was employed to detect the intima media thickness (IMT) ratio of common carotid artery of study subjects as well as controls. Lipid peroxidation was detected by serum oxidized LDL (ox-LDL) levels.

Results: The mean of carotid artery intima thickness in group A was 0.43±0.02, the mean intima thickness of group B samples was 1.0±0.09. The mean ratio of Ox-LDL/LDL in group A was 0.003±0.001. While in group B the mean of Ox-LDL: LDL was 0.012±0.002. Ox-LDL was found to have a positive linear relationship with mean carotid artery intima media thickness ratio (r=0.8) in haemodialysis group.

Conclusion: Ox-LDL to LDL ratio is much enhanced in patients on maintenance haemodialysis showing increased lipid peroxidation. Ox-LDL has a positive linear relationship with mean carotid artery intima media thickness ratio which is a marker of sub clinical atherosclerosis. Early detection of elevated Ox-LDL will help nephrologists in early detection and treatment of sub clinical atherosclerosis.

Key Words: Atherosclerosis, haemodialysis, lipid peroxidation, oxidized LDL,

How to cite this article: Rehman S, Kumar S, Nisar K, Sami Z, Mehboob F, Rehman S. Association of elevated oxidized low density lipoprotein to low density lipoprotein ratio (ox ldl/ldl) with sub clinical atherosclerosis in patients receiving maintenance haemodialysis. Ann Jinnah Sindh Med Uni. 2022;8(1):30-35

DOI 10.46663/ajsmu.v8i1.30-35

INTRODUCTION

Atherosclerosis is the major cause of mortality and morbidity in haemodialysis treated end stage renal disease patients. As estimated by European Registry of patients on renal replacement therapy, the likelihood of cardiovascular complications amongst patients on renal replacement therapy is 35-50 times more in comparison to the broad population[1,2]. To initiate the process of atherosclerosis, the LDL molecule undergoes certain biochemical modifications which initiate the typical cellular and inflammatory reactions that are characteristic of this disorder. To initiate the process of atherosclerosis, LDL can get converted into oxidized or glycated form[3,4].

Phospholipase A2 that catalyzes lipid oxidation in LDL is secreted by the inflammatory cells[5,6]. A heme protein called myeloperoxidase which is secreted by the activated phagocytes catalyzes the oxidation of L-tyrosine residue, present in the apolipoprotein B100 to tyrosyl radical. This oxidation of apolipoprotein initiates the process of lipid peroxidation in LDL molecule[7]. This results in the formation of aldehydes

1 Department of Biochemistry, Bahria University Medical and Dental College, Karachi, Pakistan

2 Jinnah Sindh Medical University, Karachi, Pakistan

3 Department of Biochemistry, Liaquat National Hospital and Medical College, Karachi, Pakistan

4 Pak Red Crescent Medical College, Kasur, Pakistan

5 Sir Ganga Ram Hospital, Lahore, Pakistan

Correspondence: Dr Sadia Rehman, Department of Biochemistry, Bahria University Medical and Dental College, Karachi, Pakistan

Email: sadia.rehman.bumdc@bahria.edu.pk

that substitute lysine residues in the apolipoprotein B-100 moiety[8]. Lipid oxidation alongwith protein oxidation in LDL results in the generation of oxidized LDL[9,10].

Oxidative Stress also leads to decreased availability of Nitric oxide which causes endothelial dysfunction[11,12]. Thus the vascular tone is directly affected. LDL cholesterol enters into the tunica intima layer of the blood vessel, where it undergoes an oxidization process and is converted into ox-LDL which is an atherogenic molecule that initiates the development and progression of vascular inflammatory process[13,14]. Other inflammatory molecules, such as macrophages, and platelets accumulate in the subendothelial area[15,16].

Several mechanisms have been proposed to explain the endothelial dysfunction and atherosclerotic plaque formation, by Ox-LDL. Initially, the induction of the expression of intercellular adhesion molecule-1 and vascular-cell adhesion molecule-1 takes place which causes the chemotactic aggregation of the macrophages and their activation and proliferation in the arterial wall, thus causing their binding to the endothelial cells of the blood vessel. The uptake of Ox-LDL by macrophages takes place leading to the formation of foam cells. The next step is stimulation of the expression of growth factors, such as fibroblast growth factor and platelet-derived growth factor by the macrophages and endothelial cells[16-18].

All these events promote the accumulation of macrophages, cholesterol, and other lipids at the site leading to formation of a plaque; hence elevated levels of Ox-LDL are associated with accelerated atherogenesis and cardiovascular disorders[19,20].

Oxidative stress induced by haemodialysis is one of the possible reasons for atherosclerotic changes in these patients[19]. In the past few years, focus has grown on the risk factors that are connected with the risk of cardiovascular events in patients with chronic renal failure such as inflammation, oxidative stress, and high C Reative Protein levels[20].

It is known that atherosclerosis represents a state of high oxidative stress characterized by the oxidation of lipids and proteins in the walls of blood vessels. Reactive oxygen species, have a causatory role in atherosclerosis and other vascular diseases[20].

This study was designed to find out the levels of oxidized Low density lipoprotein to low density lipoprotein ratio in patients receiving maintenance haemodialysis and its association with subclinical atherosclerosis. This study will help the nephrologists in identification of cardiovascular events in these patients and also help in development of treatment regimes to lower the oxidative stress in this group of patients.

METHODOLOGY

This case control, hospital based study was carried out in the Nephrology Department Ward no 22 and Radiology Department of Jinnah Post Graduate Medical Centre Karachi from January to December 2018. Ethical approval No. F.2-81-IRB/2018-GENL/5173/JPMC was obtained from the ethical review board of JPMC, Karachi. Data obtained from the study subjects was kept confidential.

Sample size was calculated from open EPI website calculator using prevalence of atherosclerosis in haemodialysis patients using a reference study carried out on haemodialysis patients to estimate intima media thickness ratio using simple random sampling technique, carried out in Shanghai China with a sample size of 84 controls and 31 subjects[21].

A sample size of 120 subjects was calculated which was further divided two groups. Group A included 60 normal controls from healthy population and group B included 60 patients receiving maintenance haemodialysis for more than two years.

Inclusion criteria was both male and female subjects with ages between 18 and 50 years, receiving haemodialysis therapy due to chronic renal failure for more than two years and not taking supplementary antioxidants. Exclusion criteria was patients suffering from malignancy, patients receiving haemodialysis due to acute renal failure, subjects having history of any previous cardiac disease or event, and all those not willing to participate in the study.

Non probability consecutive sampling technique was used for the recruitment of study subjects. Biochemical parameters were measured in both the study groups.

High resolution B mode ultrasonography was used for detection of the thickness of tunica media and tunica intima of common carotid artery.

Serum Ox-LDL levels were analyzed using CEA527Hu Human oxidized low density lipoprotein, Ox-LDL ELISA Kit.

BMI was calculated using the formula for BMI i.e. weight (kg)/height (m^2)

Data obtained was analyzed using SPSS version 23.0. The mean and standard deviation for the variables age, BMI, BP, and Lipid profiles were reported in both the

study groups. Linear regression analysis was utilized to detect relationship of carotid intima media thickness with lipid peroxidation. Independent sample t-test was utilized for the comparison of mean levels between the study groups. A P-value of = 0.05 was considered to be significant.

RESULTS

Figure 1 reports the mean age of group A participants was 34.67±7.73, mean BMI was 23.47±3.26, mean systolic blood pressure was 107.33±9.80, mean diastolic blood pressure was 66.33±8.09, mean weight was 66.10±7.75, and mean height was 1.69±0.12 meters whereas group B patients had mean age 43.20±4.66 years, mean BMI was 22.21±4.21, mean systolic blood pressure was 159.0±12.42, mean diastolic blood pressure was 93.67±10.66, mean weight was 64.67±6.13 and mean height was 1.63±0.12.

Figure 2 reports the mean comparison of serum Ox-LDL across studied groups, in group A mean Ox-LDL was 24.87±5.23 while in group B samples mean Ox-LDL was 63.77±6.77. All group mean differences were found statistically significant with p-value < 0.05.

Table 1 reports the mean and standard deviation of intima media thickness across selected patients of from the two groups, the mean of right common carotid artery of group A samples was 0.45±0.04, left common carotid artery was 0.42±0.01, and mean intima thickness was 0.43±0.02, the mean of right common carotid artery in group B was 0.93±0.09 and mean of left common carotid artery was 1.07±0.14 while the mean intima thickness of group B samples was 1.0±0.09. There was significant mean difference obtained across the studied groups for mean intima media thickness, and right, left common carotid artery outcomes with p-value < 0.01.

Table 1: Mean Comparison of Intima Media Thickness Across Studied Groups

	Group A (controls) (n=60)		Group B (haemo-dialysis group) (n=60)		p-value
	Mean	SD	Mean	SD	
Right common carotid artery -(intima media thickness mm)	0.45	0.04	0.93	0.09	<0.01*
left common carotid artery (intima media thickness mm)	0.42	0.01	1.07	0.14	<0.01*
Mean intima media thickness	0.43	0.02	1.00	0.09	<0.01*

P < 0.05 was considered as significant.

Figure 3 shows a scatter plot which estimates the correlation between oxidized LDL and mean intima media thickness ratio. A positive association of Ox-LDL with mean intima thickness was seen.

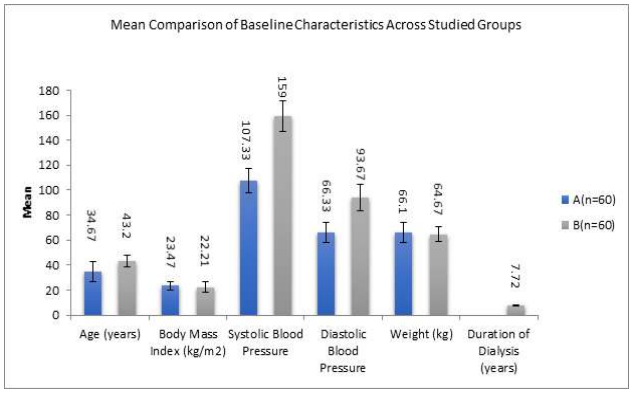

Figure 1: Comparison of Anthropometric Variables Among Study Groups

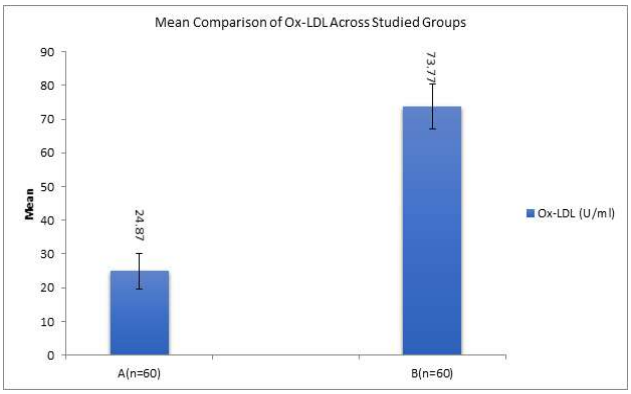

Figure 2: Mean Comparison of Ox-LDL Among Study Groups

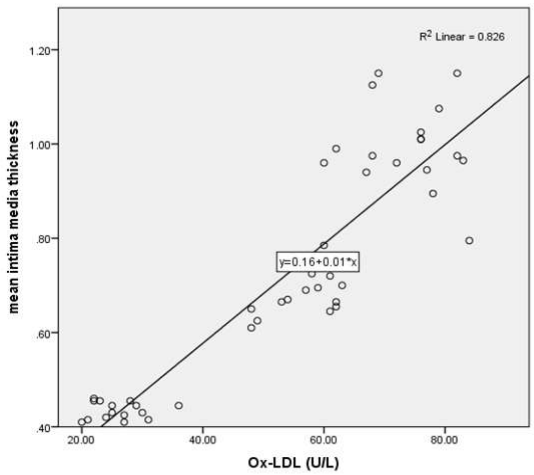

Figure 3: Correlation Between Ox-LDL and Mean Intima Media Thickness Ratio

DISCUSSION

Cardiovascular disease is the leading cause of mortality in haemodialysis patients. Oxidative stress is the major factor related to dialysis associated cardiovascular complications in these patients. In this study, we aimed to find out the correlation between oxidized low density lipoprotein to low density lipoprotein ratio with atherosclerotic changes in patients receiving maintenance haemodialysis.

No significant difference in the mean ages among the two groups was found. A significant decrease in the weight and BMI was seen between the haemodialysis patients. The decreases in BMI may be due to protein energy wasting and restricted diet in haemodialysis patients.

No significant difference in the mean ages among the two groups was found. A significant decrease in the weight and BMI was seen between the hemodialysis patients. This is similar to the findings of Rysz[22].

We found a significant increase in the mean systolic BP and diastolic BP of the haemodialysis patients as compared to the control group. This increase in BP which can be due to fluid overload, overactivity of renin angiotensin system, erythropoietin administration, and enhanced stimulation of the sympathetic nervous system. Our results are similar to the findings of Wang[23] who reported a mean SBP of 143.2 ± 32.7mmHg and mean DBP of 79.0 ± 15.9 mmHg among haemodialysis patients.

Our study showed an increase in levels of serum oxidized LDL in subjects on maintenance haemodialysis (63.77±6.77) as compared to controls (24.87±5.23). Wagner[17] reported a mean level of Ox-LDL in haemodialysis patients as 74.6 ± 28.1 U/L[14]. Hou[18] also reported increased levels of Ox-LDL (89.15± 12.3 U/L) in haemodialysis treated patients. This increase in levels of oxidized LDL is due to increased oxidative stress after multiple cycles of haemodialysis resulting in increased lipid peroxidation[16].

Significant results were obtained on Doppler ultrasonography. Significant mean difference was obtained across the two studied groups for mean intima media thickness, and right, left common carotid artery outcomes with p-value less than 0.01.

The mean of intima thickness in group A was 0.43±0.02, while the mean of intima media thickness in group B was 1.0±0.09. This signifies that patients receiving dialysis are at greater risk of developing atherosclerosis. This is in accordance with the published work of Manabe who reported a maximum IMT of = 1.5 mm in haemodialysis patients[24].

Our results are also similar to Mahmoud who also showed increased carotid artery IMT (1.0 ±0.7) in haemodialysis patients[25]. These findings suggest that patients on haemodialysis are subjected to oxidative stress that leads to the formation of oxidized LDL and generation of inflammatory mediators that lead to development of atherosclerotic heart disease. The carotid artery intima media thickness ratio is a non-invasive indicator and predictor of atherosclerotic heart disease and has been discussed in many previous studies[21].

The ox-LDL to LDL ratio was significantly raised in the haemodialysis group indicating increased oxidized fraction of LDL as compared to the native LDL in haemodialysis patients. This imbalanced ratio is because of excess lipid peroxidation in the haemodialysis patients[22,23].

Our results are similar to Mahmoud who also found that carotid artery intima media thickness is correlated with inflammatory processes in haemodialysis patients[25]. These findings suggest that patients on haemodialysis are subjected to oxidative stress that leads to the formation of oxidized LDL and generation of inflammatory mediators that lead to development of atherosclerotic heart disease. The carotid artery intima media thickness ratio is a non-invasive indicator and predictor of sub clinical atherosclerotic heart disease and has been discussed in many previous studies[24,25].

Oxidative stress in haemodialysis patients is caused due to poor dietary intake of antioxidants, formation of oxidized molecules, and loss of antioxidants during haemodialysis[25]. These factors are linked to the development of atherosclerosis and chronic inflammation and lead to cardiovascular complications in these patients. The administration of antioxidants plays a protective role against oxidative stress by neutralizing the harmful effects of oxidative molecules, however it has still not been adopted as a regular treatment protocol in clinical practice. More prospective studies are required to elaborate on the protective role of antioxidant administration in oxidative stress that can improve the cardiovascular mortality rate in haemodialysis treated end-stage renal disease. Moreover, the oxidative stress parameters in these patients need to be monitored to avoid the possible outcomes of oxidative stress. Dietary guidelines should also be developed to ensure the intake of adequate vitamins and minerals in these patients. The limitations of this study were that it was a single centered study

so the sample size was restricted, and the other parameters of oxidative stress were not monitored due to budget constraints.

CONCLUSION

Our study concluded that ox-LDL/LDL ratio is significantly elevated in haemodialysis treated patients and serves as a marker for the detection of sub clinical atherosclerosis. This study will help nephrologists in early detection and prevention of cardiovascular changes in haemodialysis patients.

Conflict of Interest: The authors declare that they have no conflict of interest.

Authors' contribution: SR: Conceived the idea and worked on research; SK: Worked on data collection and supervised the research; KN: Worked on literature review and write up; ZS: Worked on data collection and drafting; FM: Worked on literature review and final approval of draft; SR: Did statistical analysis.

REFERENCES

1. Gaæ P, Porêba M, Januszewska L, Prokopowicz A, Martynowicz H, Mazur G, et al. The Total Antioxidant Status, Serum Selenium Concentrations and the Ultrasound Assessment Carotid Intima Media Thickness in Patients with Arterial Hypertension. Antioxidants. 2021;10(1):63.
2. Asicioglu E, Velioglu A, Arikan H, Koc M, Tuglular S, Ozener C. Baseline carotid intima media thickness is associated with cardiovascular morbidity and mortality in peritoneal dialysis patients. Ther Apher Dial. 2021;25(6):962-969.
3. Abd elhamed Mohamed MA. The Relation between Hemoglobin Level Variability and Carotid Intima-Media Thickness in Chronic Hemodialysis Patients. Zagazig Uni Med J. 2020;26(5):869-76.
4. Ohtake T, Kobayashi S. Chronic Kidney Disease and Atherosclerosis: An Important Implication of Carotid Intima-Media Thickness. J. Atheroscler. Thromb. 2020:ED146.
5. Maugeri A, Hruskova J, Jakubik J, Kunzova S, Sochor O, Barchitta M, et al. Dietary antioxidant intake decreases carotid intima media thickness in women but not in men: A cross-sectional assessment in the Kardiovize study. Free Radic. Biol. Med. 2019;131:274-81.
6. Lestaringsih L, Projosudjadi W, Sya'bani M, Hadisaputro S. Correlation between the Inflammation Factors and Intima-Media Thickness in Patients with End-Stage Renal Disease (ESRD) on Regular Hemodialysis. Mol Cell Biomed Sci. 2019;3(1):48-53.
7. Cicero AF, Kuwabara M, Johnson R, Bove M, Fogacci F, Rosticci M, et al, Brisighella Heart Study group. LDL-oxidation, serum uric acid, kidney function and pulse-wave velocity: Data from the Brisighella Heart Study cohort Int J Cardiol. 2018;261:204-208.
8. Willeit P, Tschiderer L, Allara E, Reuber K, Seekircher L, Gao L, et al Brouwers FP. Carotid intima-media thickness progression as surrogate marker for cardiovascular risk: meta-analysis of 119 clinical trials involving 100 667 patients. Circulation. 2020;142(7): 621-642.
9. Furukawa S, Suzuki H, Fujihara K, Kobayashi K, Iwasaki H, Sugano Y, Yatoh S, Sekiya M, Yahagi N, Shimano H. Malondialdehyde-modified LDL-related variables are associated with diabetic kidney disease in type 2 diabetes. Diabetes Res Clin Pract. 2018;141:237-43.
10. Kerforne T, Favreau F, Khalifeh T, Maiga S, Allain G, Thierry A, et.al. Hypercholesterolemia-induced increase in plasma oxidized LDL abrogated pro angiogenic response in kidney grafts. J Transl Med. 2019;17(1):1-4.
11. Rerkasem A, Pongtam S, Ounjaijean S, Kulprachakarn K, Wongthanee A, Chaiwarith R, et.al. Ankle-Brachial Index and Carotid Intima-Media Thickness Progression by Using Ultrasound Among Patients With HIV Infection Versus End-Stage Renal Disease. Int J Low Extrem Wounds. 2020;19(4):364-8.
12. Ostadmohammadi V, Soleimani A, Bahmani F, Aghadavod E, Ramezani R, Reiter RJ,et al. The effects of melatonin supplementation on parameters of mental health, glycemic control, markers of cardiometabolic risk, and oxidative stress in diabetic hemodialysis patients: a randomized, double-blind, placebo-controlled trial. J Renal Nutr.2020;30(3):242-250.
13. Speer T, Ridker PM, von Eckardstein A, Schunk SJ, Fliser D. Lipoproteins in chronic kidney disease: from bench to bedside. Eur Heart J. 2021;42(22):2170.
14. Düsing P, Zietzer A, Goody PR, Hosen MR, Kurts C, Nickenig G, et.al. Vascular pathologies in chronic kidney disease: pathophysiological mechanisms and novel therapeutic approaches. J Mol Med. 2021;22:1-4.
15. Kirkman DL, Bohmke N, Carbone S, Garten RS, Rodriguez-Miguelez P, Franco RL. Exercise intolerance in kidney diseases: physiological contributors and therapeutic strategies. Am J Physiol Renal Physiol. 2021;320(2):161-73.
16. Liu CS, Li CI, Guo YC, Lin CH, Lin WY, Liu CH, et.al. Independent associations of urinary albumin-to-creatinine ratio and serum cystatin C with carotid intima-media thickness in community-living Taiwanese adults. BMC Nephrol. 2020;21(1):1-0.
17. Wagner S, Apetrii M, Massy ZA, Kleber ME, Delgado GE, Scharnagel H,et.al. Oxidized LDL, statin use, morbidity, and mortality in patients receiving maintenance hemodialysis. Free. Radic Res. 2017;51(1): 14-23.
18. Hou JS, Wang CH, Lai YH, Kuo CH, Lin YL, Hsu BG. Serum Malondialdehyde-Modified Low-Density Lipoprotein Is a Risk Factor for Central Arterial Stiffness in Maintenance Hemodialysis Patients. Nutrients. 2020; 12(7):2160.

19. Barati Boldaji R, Akhlaghi M, Sagheb MM, Esmaeilinezhad Z. Pomegranate juice improves cardiometabolic risk factors, biomarkers of oxidative stress and inflammation in hemodialysis patients: a randomized crossover trial. J Sci Food Agric. 2020; 100(2):846-54.
20. Raoof IB, Abdalah ME. Quality assessment of unsaturated iron-binding protein capacity in Iraqi patients undergoing hemodialysis. J Pharm Bioallied Sci. 2020; 12(3):246.
21. Li ZJ, Du LF, Qin Y, Liu JB, Luo XH. Ultrasound assessment of intima-media thickness and diameter of carotid arteries in patients undergoing emodialysis or renal transplantation. Curr Med Sci. 2018;38(4):727-33.
22. Rysz J, Franczyk B, Ławiñski J, Gluba-Brzózka A. Oxidative Stress in ESRD Patients on Dialysis and the Risk of Cardiovascular Diseases. Antioxidants. 2020; 9(11):1079.
23. Wang T, Li Y, Wu H, Chen H, Zhang Y, Zhou H, et.al. Optimal blood pressure for the minimum all-cause mortality in Chinese ESRD patients on maintenance hemodialysis. Biosci.Rep. 2020;40(8). doi: 10.1042/BSR20200858.
24. Manabe S, Kataoka H, Mochizuki T, Iwadoh K, Ushio Y, Kawachi K,et.al. Maximum carotid intima-media thickness in association with renal outcomes. J Atheroscler Thromb. 2021:28(5):5491-505.
25. Mahmoud M, Nagy E, AbdAlBary M, El-Kannishy G, Sayed-Ahmed N. Relation of protein energy wasting to carotid intima media thickness in hemodialysis patients. J. Human Hypertens. 2021;35(7):598-603.