ORIGINAL ARTICLE

Impact of Oxidative Stress on Hypertension in Patients on Maintenance Haemodialysis

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ABSTRACT

Objective: To assess the association of oxidative stress with hypertension and its correlation with the duration of haemodialysis

Methodology: It was a case control study conducted in a public sector tertiary care hospital in 2017. The study participants were recruited from the nephrology ward while the healthy controls were taken from participant neighborhood through frequency matching. Non probability consecutive sampling technique was employed. Cases included were suffering from chronic renal failure and were receiving maintenance haemodialysis. Exclusion criteria was patients suffering from any other chronic illness other than chronic renal failure (pulmonary disease and hepatic insufficiency). Detailed analysis was done with application of ANOVA, Pearson correlation and independent sample t test. P value less than 0.05 was taken as significant.

Results: Highly significant difference was observed in mean serum malondialdehyde, mean plasma superoxide dismutase, mean systolic blood presure, and mean BMI among the cases and controls (p value < 0.001). Positive linear correlation was found between blood presure and serum malondialdehyde i.e. (r = 0.4) while on the other hand strong negative correlation was found between blood presure and plasma superoxide dismutase i.e. (r = -0.73).

Conclusion: Oxidative stress worsens with progression of haemodialysis and leads to development of hypertension.

Keywords: Oxidative stress, serum malondialdehyde, hypertension, plasma SOD, BMI, haemodialysis

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اعنوان: ھیموڈاایلیسس کے مریضوں میں آکسیڈ ٹیو دباؤ کے ہائپر ٹینشن پر اثرات۔

تعارف: آکسیڈ ٹیو دباؤ کا ہائپر ٹینشن اور ھیموڈاایلیسس کے دورانیے کے ساتھ تعلق کی جانچ کرنا۔

طریقہ کار: یہ تحقیق 2017 میں ایک تیسرے درجے کے سرکاری ہسپتال میں ایک خاص کیس کی جانچ کے لیئے کی گئی۔ تحقیق میں حصّہ لینے والے افراد کا تعلق نیفرولوجی وارڈ سے تھا۔ جبکہ انکے مقابلے میں صحت مند افراد قریبی آبادی سے منتخب کیئے گئے, **Chronic renal failure** کی وجہ سے ھیموڈاایلیسس کروانے والے مریضوں پر تحقیق کی گئی۔ تفصیلی تجزیہ کے لیئے **ANOVA, Pearson correlation** اور آزاد سیمپل **t-test** کا استعمال کیا گیا جبکہ (P-value 0.05) لی گئی۔

نتائج: مریضوں اور صحتمند افراد کے درمیان سیرم **Malondialdehyde**، پلازمہ **SOD**، **Systolic BP** اور **BMI** کی اوسط قیمت میں اہم فرق (P-value<0.001) پایا گیا۔ بلڈ پریشر اور سیرم **Malondialdehyde** کے درمیان اثبت خطی تعلق (r = 0.4) پایا گیا جبکہ بلڈ پریشر اور (PSOD) پلازمہ سپر آکسائیڈ dismutase کے درمیان گہرا منفی خطی تعلق (r = -0.73) پایا گیا۔

حاصل مطالعہ: اس تحقیق سے معلوم ہوتا ہے کہ آکسیڈ ٹیو دباؤ، ھیموڈاایلیسس کے ساتھ بڑھ جاتا ہے اور ہائپر ٹینشن کا باعث بنتا ہے۔

INTRODUCTION

The main risk of mortality in patients of end stage renal disease (ESRD) is the cardiovascular diseases[1]. The US renal data system and European Registry of patients, both have highlighted that the incidence of developing a cardiac event is almost five times higher in chronic dialysis patients as compared to the healthy population[2]. Cardiovascular events in ESRD are basically a sequel of hypertension and diabetes as these two are termed number one risk factors for renal failure[3-4]. In chronic renal failure, there is a loss of equilibrium between pro-oxidant and anti-oxidant capacities and a state of increased oxidative stress is evident. The factors which are held responsible for this shift are not only cardiovascular causes but also factors which are more associated to uremia[5-11].

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The role of oxidative stress is evident in the pathogenesis of hypertension, which has high prevalence in chronic renal failure patients and is considered as the number one risk factor for the progression of cardiovascular diseases[12-13].

Oxidative stress promotes vascular smooth muscle proliferation and deposition of collagen leading to an increase in the intima media thickness ratio and hence causing narrowing of the vascular lumen[12-18]. In addition to this, oxidative stress causes imbalance between the endothelium dependent vascular relaxation and vascular contractile activity by stimulating endothelial injury and decreasing Nitric oxide availability[13-16]. All these factors contribute to the development of hypertension.

Malondialdehyde, a lipid peroxidation end product is produced as a result of the attack by the free radicals on the polyunsaturated fatty acids on the surface of the cell membranes. Malondialdehyde, if produced in high numbers, is a marker of systemic oxidation[14-18].

The term antioxidant refers to a molecule which is capable of stabilizing or deactivating free radicals before they attack normal cells[19]. Endogenous antioxidants are crucial for maintaining optimal cellular functions resulting in systemic health and well-being[20]. However, in conditions promoting oxidative stress, dietary antioxidants may be necessary to maintain the cellular function on optimal levels as endogenous antioxidants may prove to be insufficient.

Glutathione peroxidase, catalase, and superoxide dismutase are the most efficient enzymatic antioxidants. Mitochondria, being the major site of free radical generation, contain a variety of antioxidants present on both sides of their membranes in order to minimize ROS induced stress[21]. Superoxide dismutase is also among the most effective enzymatic antioxidants. Superoxide dismutases (SODs) defend against oxidative damage by enzymatically converting O_2^- to H_2O_2. According to a recent study, SOD is a major antioxidant enzyme in the regulation of oxidative stress during progressive renal injury[22].

When oxidative stress occurs, it triggers the oxidation of molecules such as lipids, proteins, and carbohydrates, leading to lipid peroxidation and accumulation of advanced glycation end products which cause severe damage to the endothelium[11-15]. Moreover, nitric oxide which causes endothelial smooth muscle relaxation is rapidly degraded by the oxygen derived free radical superoxide anion[15-20]. There is also a correlation between oxidative stress and renin activation[32]. Hence oxidative stress is involved in the pathogenesis of various conditions including hypertension, inflammation, and the progression of chronic kidney disease to end stage renal disease[12-16].

The main objective of the study was to assess the association of oxidative stress with hypertension and its correlation with duration of haemodialysis. This study emphasizes on the magnitude and complications of oxidative stress in haemodialysis patients. This study will help the nephrologists to identify oxidative stress as a major causative factor of hypertension and other cardiovascular complications and to change the typical treatment regimes by adding antioxidants to lower the oxidative stress.

METHODOLOGY

A Case Control study was conducted in the Nephrology ward of a public sector hospital of Karachi in 2017. Sample size was calculated by open epi website calculator. (**REFERENCE STUDY:** Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrology Dialysis Transplantation. 2003 Jul 1; 18 (7):1272-80.)

A sample size of 90 subjects was calculated which was further subdivided into three groups::

Group A: Healthy control group comprised 30 subjects from the neighborhood who volunteered for the study, and were matched on age, gender, and socio-economic status. Their routine laboratory investigations were within normal ranges.

Group B: Thirty subjects who had been on haemodialysis for up to three years

Group C: Thirty subjects who were on haemodialysis for more than three years

Inclusion criteria for the cases with chronic renal failure was taking haemodialysis therapy for more than two months. Exclusion criteria was the same for all the groups and comprised omission of patients with hepatic insufficiency, chronic pulmonary disease, and diabetes mellitus. Non-probability consecutive sampling technique was utilized for selection of participants. Biochemistry lab investigations and oxidative stress biomarkers were measured in all three groups. Hypertensive cutoff was taken for systolic BP 140 mmHg and for diastolic BP at 90 mmHg. Ethical permission for the present study was taken from the Institutional Review Committee, Jinnah Postgraduate Medical Centre (JPMC), Karachi diary no: NO.F.2-81-IRB/2018- GENL/5173/JPMC. Data which was obtained during the study was kept confidential.

BMI was calculated from weight and height measurements which were obtained through calibrated apparatus available in the wards. Blood pressure was measured in supine position after allowing the participant to relax for 10 minutes. Later on, 10 ml blood samples were collected before dialysis therapy in patients on haemodialysis.

The malondialdehyde (MDA) was estimated in the form of thiobarbituric acid reacting substances (TBARS) by the method of Okhawa et al, 1979. Levels of SOD were measured by using reagent method (method of Kono, 1978).

Data was entered on SPSS version 21. Mean and standard deviation were taken out for all numeric variables, whereas frequencies and percentages were taken out for categorical variables. One-way ANOVA was applied for finding difference in mean between the three groups after fulfilling the assumptions of normality and homogeneity through Shapiro Wilk test and QQ plot and Levenne test. Post hoc analysis was done through Tukey's test. Pearson correlation was applied for finding association of blood pressure with serum malondialdehyde and plasma SOD. Two sample t-test was applied for finding difference in means of serum malondialdehyde and plasma SOD on the basis of gender. P value <0.05 was taken as significant.

RESULTS

A total of n=90 participants were recruited in three groups. Males were n=55 (61%) and females were n=35 (39%). The mean age of the participants was 38±8 years.

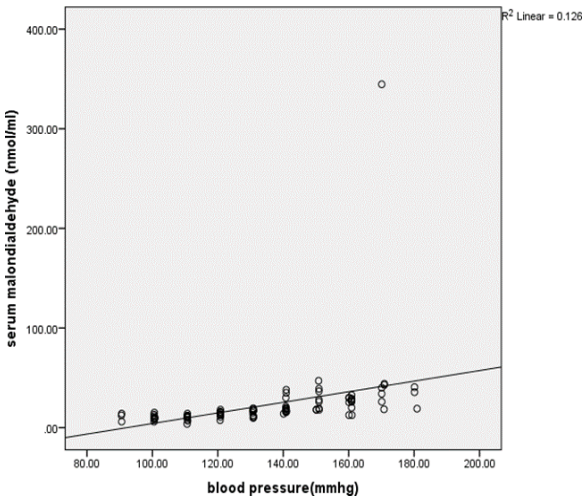
The group A comprised n=30 healthy participants. The mean age of the group was 35±7.7 years. The mean systolic BP was 108±10mmHg. Mean serum malondialdehyde (nmol/ml) was 10.87±3.04. Plasma superoxide dismutase (µ/l) was 108.5±19.4. The mean BMI of the group was 23.4±3.3 kg/m^2.

Group B, n=30 corresponded to participants who were on haemodialysis for the last three years. Mean age of the group was 36±8 years. Mean systolic blood pressure was 136±11mmHg. Mean serum malondialdehyde (nmol/ml) was 15.7±3. Plasma superoxide dismutase (µ/l) was 85±16. The mean BMI of the group was 22.6±3.7.

Group C, n=30 corresponded to participants who were on haemodialysis for more than three years. Mean age of the group was 43±4 years. Mean systolic blood pressure was 159±12mmHg. Mean serum malondialdehyde (nmol/ml) was 31±8. Plasma superoxide dismutase (µ/l) was 46±19. The mean BMI of the group was 20.8±4.6.

Table 1 summarizes the significant differences which were observed when ANOVA was applied for serum malondialdehyde, SOD, systolic BP, and BMI means difference among the three groups. Post hoc analysis was done by the Tukey HSD test. Significant differences were observed between all groups when mean serum malondialdehyde, SOD, and systolic BP was compared. Borderline significance was observed only in Group A and C when mean BMI was compared in between groups through Tukey HSD.

When serum malondialdyde was associated with systolic BP, very highly significant mild positive linear correlation was seen (r=0.4, p value<0.01) Graph 1.



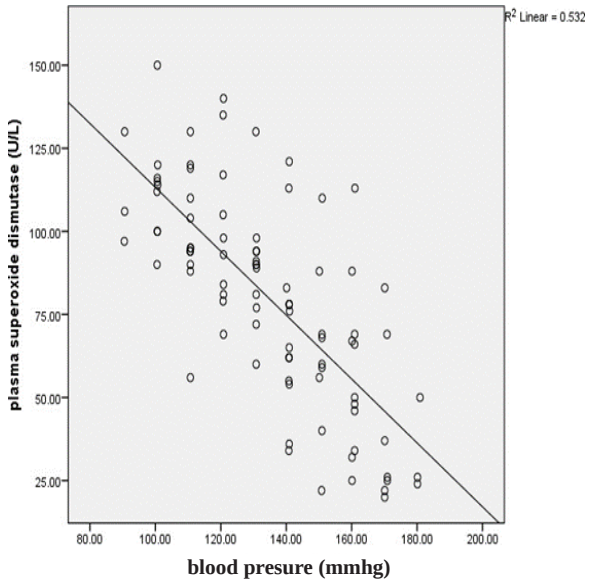
Graph 1: Association of Serum Malondialdehyde and Systolic BP

Table 1: Differences in Mean Numeric Variables Between the Three Groups (N=90)

Groups	n	SerumMalondialdehyde (nmol/ml) Mean±Std.dev	SOD Mean±Std.dev	Systolic BP Mean±Std.dev	BMI Mean±Std.dev
A	30	10.9±3.03	108.5±4	108±10	23.4±3.3
B	30	15.7±2.9	85±16	136±2	22.6±3.7
C	30	31.01±8.48	46±19	159.6±12.3	20.8±4.0
P-Value*	-	<0.01	<0.01	<0.01	0.037

* ANOVA

When superoxide dismutase (SOD) was associated with systolic BP, strong negative linear relationship was observed which was found to be very highly significant (r=-0.73, P value<0.01) Graph 2.



Graph 2: Association of Plasma SOD and Systolic BP

No significant effect of gender was observed on serum malondialdehyde and plasma SOD when independent sample t-test was applied for finding difference among these two parameters on the basis of gender.

DISCUSSION

In uremic patients, cardiovascular disease causes substantially higher morbidity and mortality than in the general population. In dialysis patients, cardiovascular mortality occurs approximately 30 times more than the risk in the general population. Even after stratification for age, gender, and presence of diabetes, this rate remains 10 to 20 times higher. Hypertension is associated with increased oxidative stress. During the comparison of study variables between the controls and patients on haemodialysis, statistically significant difference was found. Significant difference was not found in the mean ages among the three groups compared at 95% confidence interval.

The current study revealed a statistically significant decrease in weight and BMI among cases and controls which is similar to the findings of Larumbe et al[23]. This is in contrast to Araújo et al who reported an increased BMI in females undergoing dialysis[24].

In our study, we found significant increase in mean systolic and diastolic blood pressure of cases as compared to controls, which might be due to ROS induced vasoconstriction and vascular damage, decreased nitric oxide and antioxidant bioavailability, hypervolemia, renin angiotensin system overactivity, erythropoietin administration, and increased sympathetic stimulation. Bansal et al. also reported an increase in mean systolic and diastolic blood pressure in haemodialysis patients[25].

Serum malondialdehyde was significantly increased in group B and group C as compared to controls. This supports our previous findings indicating that oxidative stress in haemodialysis increases as the duration of therapy increases and plays a role in lipid peroxidation leading to development of atherosclerosis and hypertension. The possible mechanism which leads to increase in MDA levels is that ROS activate phospholipase A2 causing peroxidation of many mediators by arachidonic acid which are finally metabolized to MDA. Increased concentrations of malondialdehyde lead to formation of oxidized LDL which plays a major role in the development of atherosclerosis.

Our study further shows that mean SOD levels were significantly decreased in haemodialysis patients as compared to controls. Our results are in accordance with previous studies of Ushanthika et al and Celik et al[27]. Very low levels of SOD were found in group C. The possible mechanism behind this low level depends on several factors, such as age, creatinine clearance, uremic state, dialysis period, selective permeability of the dialyzer membrane to antioxidants and the bacterial contaminants from the dialysate[28]. (Okhawa H et al) In contrast to our study Ninia et al. showed increased levels of SOD in haemodialysis patients[29].

Oxidative stress is a universal challenge in haemodialysis patients. The enhanced oxidative stress status that characterizes haemodialysis patients, mainly occurs because of a diet lacking exogenous antioxidants, accumulation of oxidative products, and loss of antioxidant molecules during haemodialysis. Development of hypertension, chronic inflammation, and CVD mortality is also highly linked to it. It is yet to be made a part of everyday clinical practice even though administering antioxidants appears to be beneficial against oxidative stress development in maintenance haemodialysis patients. Large, prospective studies are urgently needed to elucidate the possible protective role of antioxidant administration against cellular stress that hold the promise to ameliorate the cardiovascular risk profile in CKD and 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 strength of our study was the comparison of two groups of dialysis with healthy controls. However, the limitation was the small sample size with selection of study participants through probability sampling technique.

CONCLUSION

The study results have clearly demonstrated a significant increase in oxidative stress marker (malondialdehyde) and a decrease in the antioxidants in patients receiving maintenance haemodialysis as compared to controls. The oxidative stress increases as the duration of dialysis increases showing a positive correlation with hypertension.

Authors' contributions: Dr Sadia Rehman conceived the study, searched for literature, contributed in data collection, analysis and review, and worked on introduction and discussion. Dr Santosh kumar and Dr Abdul Manan worked on literature search, results and discussion. Dr Fatima Mehboob and Dr Hasan Ali reviewed the literature, contributed to the discussion and edited the manuscript. Dr Noor un Nisa reviewed the literature, results and conclusion. All authors contributed to the final manuscript.

References

1. Agarwal R, Flynn J, Pogue V, Rahman M, Reisin E and Weir MR. Assessment and management of hypertension in patients on dialysis. J Am Soc Nephrol. 2014; 25(8):1630-1646
2. Alam A, Amanullah F, Baig-Ansari N, Lotia-Farrukh I, Khan FS. Prevalence and risk factors of kidney disease in urban Karachi: baseline findings from a community cohort study. BMC Res Notes. 2014; 7(1):179
3. Arici M and Walls J. End stage renal disease, atherosclerosis and cardiovascular mortality: is C-reactive protein the missing link? Kidney Int. 2001; 59(2):407-414
4. Asamiya Y, Yajima A, Tsuruta Y, Otsubo S and Nitta K. Oxidised LDL/LDL-cholesterol ratio and coronary artery calcification in haemodialysis patients. Nut Metab Cardiovas Dis. 2013; 23(7):619-627
5. Celik G, Capraz I, Yontem M, Bilge M, Unald M and Mehmetoglu I. The relationship between the antioxidant system oxidative stress and dialysis-related amyloidosis in hemodialysis patients. Saudi J Kidney Dis Transplant. 2013; 24(6):1157-1164
6. Chobanian AV. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute. Hypertension, 2003; 42:1206-52
7. Coombes JS, Fassett RG. Antioxidant therapy in hemodialysis patients: a systematic review. Kidney Int. 2012; 81(3):233-46
8. Cozzolino M, Galassi A, Pivari F, Ciceri P, Conte F. The Cardiovascular Burden in End-Stage Renal Disease. In Expanded Hemodial. 2017; 191:44-57
9. Dai L, Golembiewska E, Lindholm B, Stenvinkel P. End-stage renal disease, inflammation and cardiovascular outcomes. In Expanded Hemodial. 2017; 191:32-43
10. Descamps-Latscha B, Drüeke T, Witko-Sarsat V. Dialysis-induced oxidative stress: biological aspects, clinical consequences, and therapy. In Seminars Dial. 2001; 14(3):193-199
11. Förstermann, U, Xia N, and Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circulation Res. 2017; 120(4):713-735
12. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal. 2011; 15(6):1583-606
13. Furukawa S, Suzuki H, Fujihara K, Kobayashi K, Iwasaki H, Sugano Y and Shimano H. Malondialdehyde-modified LDL-related variables are associated with diabetic kidney disease in typoe 2 diabetes. Dia Res Clin Prac.2018; 141:237-243
14. Himmelfarb J. Hemodialysis complications. American Journal of Kidney Diseases. 2005; 45(6):1122-31
15. Khoubnasabjafari M, Ansarin K, Jouyban A. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. BioImpacts: BI. 2015; 5(3):123
16. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophy. 1978; 186(1):189-195
17. Kuchta A, Pacanis A, Kortas-Stempak B, Çwiklinska A, Zietkiewicz M, Renke M, Rutkowski B. Estimation of oxidative stress markers in chronic kidney disease. Kidney and Blood Press Res. 2011; 34(1):12-19
18. Kundoor N, Mohanty S, Narsini RK and Kumar TN. Pro-oxidants and antioxidants levels in chronic renal failure patients treated by dialysis. Asian J Pharmaceu Res Health Care; 2017; 9(2):71-74
19. Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR. Oxidative stress in hemodialysis patients: A review of the literature. Oxid Med Cell Longev. 2017; 2017:3081856
20. Libetta C, Sepe V, Esposito P, Galli F, Dal Canton A. Oxidative stress and inflammation: implications in uremia and hemodialysis. Clin Biochem. 2011:31; 44 (14- 15):1189-98
21. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant. 2003; 18(7):1272-80
22. Marjani A, Velayeti J, Mansourian AR and Dahmardeh N. Evaluation of oxidative stress and thyroid hormone status in hemodialysis patients in Gorgan. Indian J Physiol Pharmacol. 2017; 61(3):278-284

23. Modaresi A, Nafar M, Sahraei Z. Oxidative stress in chronic kidney disease. Iran J KidneyDis. 2015; 9(3):165-179

24. Mollace V, Gliozzi M, Musolino V, Carresi C, Muscoli S, Mollace R,et.al. Oxidized LDL attenuates protective autophagy and induces apoptotic cell death of endothelial cells: Role of oxidative stress and LOX-1 receptor expression. Intern J Cardiol. 2015; 184:152-58

25. Nakamura Y, Inagaki M, Kenmotsu S, Yamadera S, Ohsawa I, Gotoh H et.al. Significance of Cu/Zn-Superoxide dismutase levels in hemodialysis patients: A mini review. Modern Res Inflam. 2017; 6(2):9-13

26. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. Rsc Advances. 2015; 5(35):27986-8006

27. Ninic A, Sopic M, Munjas J, Spasojevic-Kalimanovaska V, Kotur-Stevuljevic J, Bogavac-Stanojevic N et.al. Association between superoxide dismutase isoenzyme gene expression and total antioxidant status in End-Stage renal disease patients on hemodialysis. Balkan Med J. 2018; 35(6):431-436

28. Okhawa H, Ohishi N, Yagi K. Reaction of linoleic acid hydroperoxides with thiobarbituric acids. Anal Biochem. 1979; 95:351-354

29. Perrotta I, Aquila S. The role of oxidative stress and autophagy in atherosclerosis. Oxid Med Cell Longev. 2015; 2015: 130315

30. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem. 2015; 97:55-74

31. Popolo A, Autore G, Pinto A, Marzocco S. Oxidative stress in patients with cardiovascular disease and chronic renal failure. Free Radic Res. 2013; 47(5):346-56

32. Pickering TG. Diagnosis and evaluation of renovascular hypertension: Indications for therapy. Circulation. 1991;83(2):I147–54