www.jmscr.igmpublication.org *Impact Factcor-1.1147*
ISSN (e)-2347-176x

IGM Publication Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Effect of Hemodialysis on Oxidative Stress on Diabetogenic Patient of Chronic Renal Failure

Authors

Ashish Shukla[1], Akash Gupta[2*], Naved Ahmad[3]

1 Assistant Professor, Department of Biochemistry, Subharti Medical College, Meerut, U.P.

2* Assistant Professor, Department of Biochemistry, Rama Medical College, Hapur, U.P.

3 Phd Scholar, Department of Biochemistry, Subharti Medical College, Meerut, U.P.

*Corresponding author

Dr Akash Gupta

Department of Biochemistry,

Rama Medical College

Pilakhua -245304, Hapur, U.P. India

Email - *akash_inspace@yahoo.com*

Abstract

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism. Advance glycations end products in diabetes also give rise to free radicals, which shift the balance in favour of prooxidant resulting in oxidative stress which is present in diabetogenic chronic renal failure patients undergoing hemodialysis. Hemodialysis can control uremia, reduce hypotensive episode, resolves acute renal failure rapidly and lowers the mortality significantly. The objective of the study was to estimate and compare markers of oxidative stress (Malondialdehyde, Glutathione peroxidise and Vitamin C) in pre and post hemodialysis patients. Fifty diabetic patients diagnosed as per criteria of American Diabetic Association, under going hemodialysis, were selected for the study after obtaining the ethical clearance from the institute. Levels of MDA, GPx and Vitamin C were estimated before and after hemodialysis. We observed that MDA levels were significantly raised in post hemodialysis as compared to pre

hemodialysis (p < 0.001) while glutathione peroxidise and vitamin C levels were significantly lower in post hemodialysis as compared to pre hemodialysis. As their is consumption of antioxidants by over production of free radicals/ reactive oxygen species in hemodialysis, levels of malondialdehyde, glutathione peroxidise and vitamin C can lead us more close to new approaches for improvement in hemodialysis membranes and its techniques and also exogenous supplementation of antioxidant for removal of free radicals.

Key words – Free radicals, MDA, GPx, Vitamin C, Hemodialysis,

Introduction

Kidney disease in diabetic patients is clinically characterized by increasing rate of urinary albumin excretion starting from microalbuminuria to macroalbuminuria to end stage renal disease (ESRD). Microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease at which appropriate interventions can retard, or reverse, the progress of the disease. According to Diabetes Atlas 2006 1, India has an estimated 40.9 million diabetic patients by 2007.

Free radicals reactions including lipid peroxidation are considered to be important factors in the pathogenesis of variety of disease2,3. They are generated due to advance glycation end products (AGEs) in diabetes which shift the balance in favour of pro oxidant resulting in oxidative stress4. Due to the action of the free radicals, the lipids can undergo the highly damaging chain reaction of lipid peroxidation (LP) which is detrimental to the functioning of the cell5. The process of lipid peroxidation give rise to may products like malondialdehyde (MDA), 4-hydroxynonenal (4-HNE) and various 2-alkenals. MDA is a 3-Carbon molecule with two aldehyde groups with formula CH2 (CHO) 2 which renders it highly reactive with other biomolecules. Hence measuring the levels of MDA in biological samples is a measure of oxidative stress6. Glutathione Peroxidases (GPx) are antioxidant enzymes7. It plays a critical role in the reduction of lipid and hydrogen peroxide8. It interacts with complex lipids such as cholesterol and lipoproteins damaged by free radicals9. Protective effect of GSH against the oxidative breakdown of lipids is mediated through GPx.

Vitamin C is a potent reducing agent as it donates electrons to recipient molecule10. Related to this oxidation-reduction potential, two major function of Vitamin C are as antioxidant and as an enzyme cofactor11.

Hemodialysis (HD) done for patients of CRF, relies on the principle of solute diffusion across a semi permeable membrane. Daily dialysis may better control uremia, reduce hypotensive episode, more rapidly resolve acute renal failure and significantly lower mortality. HD has been found in many studies as a potent source of oxidative free radical12. There is increasing evidence about the presence of oxidative stress in CRF patients, and particularly in those submitted to HD therapy. This could be due to membrane material which activates endogenous inflammatory mechanism and increased production of ROS13, 14. An imbalance between the productions of ROS and

various antioxidant defences result in oxidative stress and may be implicated in HD related complications14. Hence we decided to estimate pre and post HD oxidative stress levels by assessing MDA, GPx and Vitamin C in diabetogenic CRF patients.

Materials and methods

The present hospital based study was under taken in the Department of Biochemistry in collaboration with the Department of Medicine of Subharti Medical College. After obtaining clearance of ethical committee of the institution the work was started in 2011 and completed in 2013. A total of 50 patients diagnosed to be having diabetes as per the criteria laid down by American Diabetes Association, 2007; and chronic kidney disease as per the NKF-DOQI clinical practice guidelines for CKD1, undergoing hemodialysis, at the dialysis unit of the hospital were included in the study.

Patients with blood pressure > 130/80 mm Hg and patients who were smokers and alcoholics were excluded from the study.

Blood samples were taken twice from the HD patients, immediately before (Pre HD) and after (post HD) dialysis sessions from arterio venous fistulas (AVF) in tubes with and without anticoagulants (EDTA and Heparin). Samples were immediately centrifuged at 3000 RPM for 5 minutes. Plasma and serum samples were stored at -80ºC and kept till analysis. Plasma sample was used for determination of MDA and Heparinized whole blood was used for measurement of GPx. MDA was quantified by OxiSelect TBARS assay kit15 on double beam UV/ Vis spectrophotometer. GPx was estimated by assay kit from Randox 16 on semiautoanalyzer. Vitamin C was estimated by colorimetry using Lowry, Lopez and Bessey method17.

Statistical analysis

Data was analysed using the students paired t test. The data for biochemical analysis were expressed as mean ± SD and p value of < 0.05 was considered as significant.

Results

Age and sex wise distribution of the patients is shown in Table 1.

Table 1 – Age and Sex distribution of patients

		Total patients n=50
Age (mean ± SD)		54.8 ± 11.3
Sex	Male	39
	Female	11

The average age for patients undergoing HD was 54.8 ± 11.3 years. In our study the number of male

patients was more as compared to female who underwent hemodialysis for renal failure. There were no significant gender differences in the values of all the parameters before and after hemodialysis. When applying students paired t test before and after the hemodialysis following facts were observed. The values of MDA were significantly raised in post HD patients when compared with pre HD patients ($p < 0.01$) and both the values were higher than the normal reference range. The values of GPx were significantly low in post HD patients when compared with the values before HD and both the values were higher than the normal reference range. We also observed that the levels of vitamin C were significantly lower in post HD patients when compared with pre HD patients but the values of vitamin C were within the normal range (Table 2)

Table 2 – Mean ± SD values of different parameters of patients undergoing hemodialysis

Parameters	Patients on Hemodialysis (HD) n=50		P value
	Before HD	After HD	
MDA (nmol/ml)	2.03 ± 0.3	2.93 ± 0.2	< 0.01
GPx (U/L)	5342.05 ± 531.12	4866.55 ± 442.91	<0.01
Vitamin C (mg/dl)	1.67 ± 0.17	1.10 ± 0.15	<0.01

Discussion

Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications 18. Antioxidant mechanism that serve as a safeguard against highly reactive oxygen radicals seem to be impaired in HD patients. Dialysis can directly affect anti-oxidant nutrient status and thus indirectly contribute to oxidant stress10.

In the present study we examined the effect of hemodialysis on oxidative stress in patients of diabetogenic CKD using selected markers. Various other studies have also found similar results. Study of Samouilidou E et al19 in 31 HD patients and 17 controls showed that plasma MDA of HD patients increased in the post HD group when compared with pre dialysis and control group. It was found that before HD, TCA and MDA levels were higher than those in the NC ($p<0.001$). After HD, these levels decreased significantly but were higher than in NC and lower than in PD patients ($p<0.001$). The levels of 4-hydroxy alkenals were elevated in patients as

compared with NC, but did not differ between HD and PD patients. The MDA concentrations correlated positively with the TAC in the patients.

Meltem Ozden et al7 in their study showed that the plasma MDA levels to be increased in post hemodialysis and continuous ambulatory peritoneal dialysis patients.With respect to antioxidants glutathione levels were significantly lower in pre hemodialysis, post hemodialysis and CAPD groups then those in control group. While erythrocytes GSH levels were significantly lower in the prehemodialysis patients then those in post hemodialysis and CAPD patients ($p<0.0001$), it was significantly lower in post hemodialysis patients then those in CAPD patients ($p<0.05$). There was no significant differences with respect to erythrocyte GPx levels among the groups.($p>0.05$). In a study by Montazerifer et al20 showed that MDA significantly increased after HD while GPx decreased after HD. They also found that there was a significant negative correlation between GPx and MDA.

Varan et al 18 in their study proved that increased oxidative stress in HD patients may arise from uremia, associated metabolic/humoral abnormalities and the bio incompatibility of the HD procedure. No significant differences was observed in serum GSH levels and Vitamin E levels between the groups, however, the Vitamin C level was found to be higher in the HD group. Antioxidant enzyme SOD and GSH-Px, were lower in patients undergoing HD compared with controls, as opposed to the CAT level which was found to be higher in HD patients. Serum GSH and Vitamin C levels were found to be decreased, where as no change was observed in Vitamin E levels. As for the antioxidant enzymes, SOD, CAT and GSH-Px levels were all significantly increased at the end of the HD session. Clermont G et al13 found that Vitamin C level were lower in post dialysis patients when compared with non-dialysis patients and healthy controls.

As many of the studies were done in different parts of the world so we conducted this study in this part of India, to find that is there any geographical or racial variation which occurs in oxidative stress due to hemodialysis. But we found it to be in accordance with other studies conducted by researchers in different regions of the world.

Conclusion

Our result suggest an increase in ROS accompanied by decreased antioxidant defence in CRF patients on hemodialysis as shown by raised level of MDA and a decline in GPx and vitamin C levels. To compensate for dialytic losses and consumption of antioxidant by over production of ROS, new approaches to dialysis membrane composition, hemodialysis technique and usage or different exogenous supplementation of antioxidant for removal of ROS, are important. Our study can lead to minimize the adverse effect of the hemodialysis procedure.

References

1. Sicree R, Shaw J, Zimmet P: Diabetes and impaired glucose tolerance. In Diabetes Atlas. 3rd ed. Gan D, Ed. Kortrijik (Heule), Belgium, International Diabetes Federation. 2006,p.15-103.
2. Morena M, Cristol JP, Canaud B. Why hemodialysis patients are in a prooxidant state? What could be done to correct the proantioxidant imbalance. Blood Purif 2000;18(3):191-199.
3. Mayer B, Zitta S, Greilberger J. Effect of hemodialysis on the antioxidant properties of serum. Biochim Biophys Acta. 2003;1638(3):267-72.
4. Vlassara H, Striker GE. AGE restriction in diabetes mellitus: a paradigm shift. Nat Rev Endocrinol. 2011;7(9):526-539.
5. Devasagayam TPA, Boloor KK, Ramsarma T. Methods for estimating lipid peroxidation: Analysis of merits and demerits (Minireview). Indian J of Biochemistry and Biophysics. 2003;40(5):300-308
6. Farmer EE, Davoine C. Reactive electrophile species. Curr Opin Plant Biol. 2007;10(4):380-386.
7. Ozden M, Maral H, Akaydin D, Cetinalp P, Kalender B. Erythrocyte glutathione peroxidase activity, plasma malondialdehyde and erythrocyte glutathione levels in haemodialysis and CAPD patients. Clin. Biochem.2002;35 (4) :269-73.
8. Imai H, Nakagawa Y. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. Free Radic Biol Med. 2003;34(2):145-169
9. Arthur JR. The Glutathione peroxidases. Cell Mol Life Sci. 2002;57:1825-1835.
10. Coombs J, Gerald F. The Vitamins. 4ed. Burlington: Elsevier Science; 2012
11. Erdman JW, MacDonald I, Zeisel SH. International Life Sciences Institute. Present knowledge in Nutrition . 10th ed. Ames, Iowa: 2012
12. Clermont G, Lecoura S, Lahet JJ, Siohan P, Vergely C, Chevet D et al. Alteration in plasma antioxidant capacities in chronic renal failure and hemodialysis patients: a possible explanation for the increased cardiovascular risk in these patients. Cardiovascular Research. 2000;47(3):618-623
13. Gastaldello K, Husson C, Wens R, Vanherweghem JL, Tielemans C. Role of complement and platelet-activating factor in the stimulation of phagocytosis and reactive oxygen species production during hemodialysis. Nephrol DialTransplant 2000;15:1638–46.
14. Ward RA, Ouseph R, McLeish KR. Effects of high-flux hemodialysis on oxidant stress. Kidney Int 2003;63:353–359.

15. OxiSelect TBARS MDA estimation protocol. Available from: http://www.cellbiolabs.com/sites/default/files/STA-330-tbars-assay-kit.pdf
16. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase. J Lab Clin Med. 1967;70:158-169.
17. Packer JE, Slater TF, Wilson RL: Direct observation of a free radical interaction between vitamin E and vitamin C. Nature. 1979;278(5706):737-738.
18. Varan HI, Dursun B, Dursun E, Ozben T, Suleymanlar G. Acute effects of hemodialysis on oxidative stress parameters in chronic uremic patients: comparison of two dialysis membrane. Ind J Nephrol Renovasc Dis. 2010;3:39-45.
19. Samouilidou E, Grepsa E. Effect of dialysis on plasma total antioxiadant capacity and lipid peroxidation products in patients with end stage renal failure. Blood Purifi. 2003;21(3):209-212.
20. Montazerifar F, Hashemi M, Karajibani M, Sanadgol H, Dikshit M. Evaluation of lipid peroxidation and erythrocyte glutathione peroxidise and superoxide dismutase in hemodialysis patients. Saudi J Kidney Dis Transpl. 2012;23(2):274-279.