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Original Research Article

Study of Oxidative Stress Markers and Total Antioxidant Status in Pre and Post Hemodialysis Session among Chronic Kidney Disease Patients

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Abstract:

Introduction: Chronic renal failure is associated with increased inflammation and oxidative stress which is responsible for the development of cardiovascular disorder and it represent leading cause of death in chronic renal failure patient. The present study was undertaken to know whether there is hemodialysis induced generation of reactive oxygen species with further decrease in total antioxidant status and to compare the biochemical parameters in pre and post hemodialysis with that of healthy controls.

Aims and Objectives: (1). To study hsCRP, Malondialdehyde (MDA), lipid profile, urea, creatinine, uric acid levels in pre and post hemodialysis session of chronic kidney disease patients. (2). To find the association of oxidative stress with total antioxidant status in pre and post dialysis among chronic kidney disease patients.

Materials and Methods: In our study blood samples were collected from 30 chronic kidney disease patients in one dialysis session both before and after hemodialysis. This study was conducted at ACSR Government Medical college.

Results and Discussion: We could establish highly significant (P value ≤ 0.001) increase in hsCRP, MDA values corrected to serum creatinine in post-dialysis in comparison to pre dialysis in our study. The decrease in mean values of urea, uric acid and creatinine after dialysis was highly significant in comparison to predialysis samples. There was decrease in FRAP in postdialysis when compared with predialysis and this was statistically highly significant. We could not establish any association of oxidative stress with total antioxidant status in our study.

Keywords: Chronic Kidney disease (CKD), Pre Hemodialysis (Pre HD), Post Hemodialysis (Post HD), Total Antioxidant status (TAS), Ferric Reducing Antioxidant Power (FRAP).

Introduction

Chronic kidney disease (CKD) is a progressive loss in renal function over period of many months or years. There is decline in nephron function and number generally quantitated as reduction in glomerular filtration rate (GFR). [1] The Kidney Disease Outcomes Quality Initiative (KDOQI) of the National Kidney Foundation (NKF) guidelines defines CKD as either kidney damage or a decreased glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2 for at least 3 months. [2]

Chronic renal failure is associated with increased inflammation and oxidative stress which play an important role in the development of cardiovascular disorder and it represents leading cause of death in chronic renal failure patients [3] Oxidative stress, endothelial dysfunction and inflammation play an important role in development and progression of atherosclerosis. Gradual increase in oxidative stress has been reported with increasingly longer durations of dialysis therapy, further suggesting that oxidative stress could accelerate renal injury progression by inducing cytotoxicity. [4] Oxidative stress arises due to the imbalance between the reactive oxygen and nitrogen species and impairment of antioxidant defense mechanism. [5]

C-reactive protein (CRP) is not only biomarker of inflammation as it has been found in atherosclerotic plaques and shown to be cause of endothelial cell dysfunction, oxidant stress in experimental model. Hemodialysis may induce a state of inflammation and increase in hs-CRP may be the result of inflammatory response suggesting that dialysis procedure with its extracorporeal circulation of blood, may itself be the cause of inflammation [6] Increased oxidative stress occurs in hemodialysis patients and is dependent on many factors such as

aging, uremia, loss of residual renal function and those on regular dialysis. [7] The present study is undertaken to know whether there is hemodialysis induced generation of reactive oxygen species with further decrease in total antioxidant status and to compare the biochemical parameters in pre and post hemodialysis with that of healthy controls.

Aims and Objectives

Aim: To do a study on oxidative stress markers and total antioxidant status in pre and post hemodialysis session among chronic kidney disease patients.

Objectives:

1. To study hsCRP, Malondialdehyde (MDA), lipid profile, urea, creatinine, uric acid levels in pre and post hemodialysis session of chronic kidney disease patients
2. To study total antioxidant status by ferric reducing antioxidant power method (FRAP)among pre and post dialysis session group
3. To find the association of oxidative stress with total antioxidant status in pre and post dialysis among chronic kidney disease patients.

Materials and Methods

The aim of the study was to evaluate the association of hsCRP with total antioxidant status in pre and post hemodialysis session among chronic kidney disease patients.

The study was conducted at ACSR Government medical college, Nellore for a period of two months in 2020. The present study included 30 CKD patients on maintenance hemodialysis in the dialysis unit of ACSR government general hospital and 30 healthy controls were included in the study.

Blood samples were collected from cases in two sessions. One sample before the hemodialysis (pre-HD) and second sample on the same patient post dialysis (post – HD). The biochemical parameters hsCRP, Malondialdehyde (MDA), urea, creatinine, uric acid and FRAP were measured in both the samples and compared with that of control group.

Informed and written consent was taken from all subjects. Institutional ethical committee clearance was obtained.

Inclusion Criteria: Subjects diagnosed with chronic kidney disease and should have been on hemodialysis for more than 3 months.

Exclusion Criteria: Patients with chronic inflammatory conditions, less than 3 months on hemodialysis and those suffering with HIV or HBS Ag were excluded from the study. 5ml of blood was collected and serum separated and preserved at -20^0c until further analysis. In all the patient samples, serum urea, creatinine, , uric acid, hsCRP, FRAP and MDA were estimated using commercially available kits. The mean values compared and analyzed.

The mean and standard deviation were calculated for all the Biochemical parameters. The significance between the groups were determined using Student t- test for Equality of means. The p-value of < 0.05 was considered significant.

Results

Comparison of Mean and S.D values biochemical parameters during pre and post dialysis session

In the present study the mean value ± SD of serum urea in predialysis samples (n=30) were 103.5 ± 6.06 mg/dl and in post-dialysis samples (n=30) were 54.9± 4.71 mg/dl respectively. This increase was statistically significant ($p<0.0001$) as shown in table (1). In the present study the mean values of serum creatinine in predialysis samples were 10.44 ± 0.63 mg/dl and in post-dialysis were 5.51 ± 0.47 mg/dl.

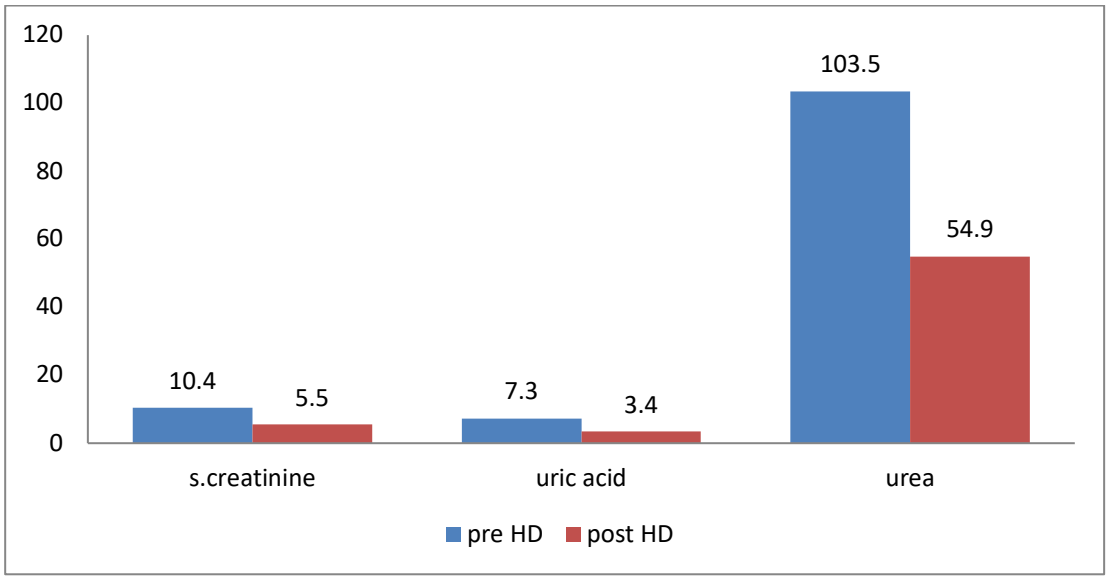
The results were statistically highly significant ($p<0.001$) as shown in table (1) the mean value and standard deviation of serum uric acid in predialysis (n=30) and post dialysis cases (n=30) were 7.3 ± 0.26 mg/dl and 3.42 ± 0.27 mg/dl respectively. The mean serum uric acid levels were statistically highly significant ($p<0.0001$) as shown in table (1).

The mean value and standard deviation of serum hsCRP in pre and post dialysis cases were (n=30) were 4.7 ± 0.3 mg/L and 5.1 ± 0.3 mg/L respectively. This increase was statistically significant ($p<0.0001$) as shown in table (1). The mean value and standard deviation of FRAP in controls (n=50) and pre-eclamptic cases (n=50) were 0.66 ± 0.02 U/ml and 0.45± 0.02 U/ml respectively. This decrease was statistically significant ($p<0.0001$) as shown in table (1).

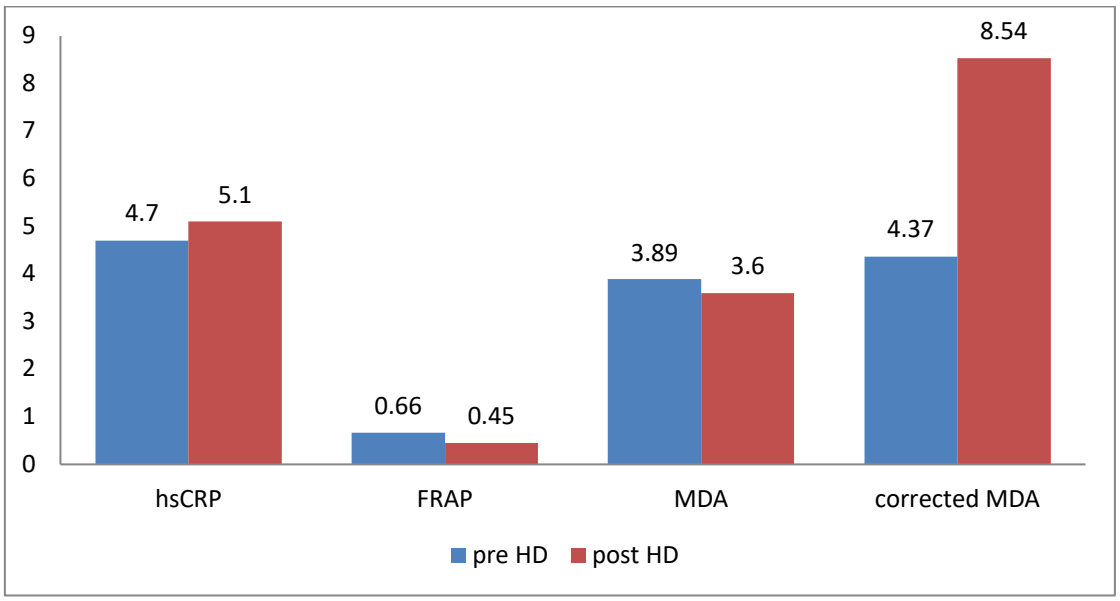
The mean value and standard deviation of MDA in predialysis session (n=30) and post dialysis session (n=30) were 3.89± 0.22 µmol/l and 3.60± 0.21 µmol/l respectively. This decrease was statistically significant ($p<0.0001$) as shown in table (1). The mean value and standard deviation of corrected MDA (µmol/mg of creatinine) in predialysis session (n=30) and post dialysis session (n=30) were 0.0436± 0.0042 µmol/l and 0.0854 ± 0.0115 µmol/mg respectively. This increase was statistically significant ($p<0.0001$) as shown in table (1).

Table 1: Mean± S.D, P values of urea, creatinine, hsCRP, FRAP and MDA and corrected MDA

S. No.	Parameter	Predialysis samples		Post dialysis samples		T value	p-value
		Mean	S.D	Mean	S.D		
1	Urea mg/dl	103.5	6.0	54.9	4.7	34.92	<0.0001
2	Creatinine mg/dl	10.44	0.6	5.5	0.47	34.35	<0.0001
3	Uric acid mg/dl	7.30	0.26	3.42	0.27	56.69	<0.0001
4	hsCRP mg/dl	4.7	0.3	5.1	0.3	5.16	<0.0001
5	FRAP U/mL	0.66	0.02	0.45	0.02	40.66	<0.0001
6	MDA µmole/L	3.89	0.22	3.60	0.21	5.2	<0.0001
7	Corrected MDA µmole/mg creatinine	0.0437	0.004	0.085	0.0115	20.34	<0.0001



Graph 1: Comparison of mean serum creatinine, uric acid and urea in pre and post dialysis cases (P-value significant)



Graph 2: Comparison of mean serum hsCRP, FRAP, MDA and corrected MDAin pre and post dialysis cases (P-value significant)

Discussion

Chronic kidney disease is a progressive loss in renal function over period of many months or years. There is decline in nephron function and number generally quantitated as reduction in glomerular filtration rate. [1] Chronic kidney disease is a global problem which should be

diagnosed at an early stage so that an immediate intervention can be undertaken and its progression to end stage renal failure can be circumvented. [8]

In our study 30 chronic kidney disease patients undergoing hemodialysis were enrolled and blood samples collected from them one sample before dialysis (predialysis/preHD) and one sample after dialysis. (Post dialysis/post HD).

In our study the mean values of urea and creatinine in post-dialysis session was decreased significantly in comparison to pre dialysis. [P value <0.0001, table no.1, Graph No 1). R.Nisha, srinivasa kannan et al [9] and Amin N, Raja Tahir Mohammad et al [10] in their studies have showed significant reduction of urea and creatinine levels post hemodialysis. This was in accordance to our study.

The mean values of uric acid in our study decreased significantly during post dialysis in comparison to pre-dialysis [P value <0.001, table no.1, Graph No 1).

In our study the decrease in mean values FRAP during post dialysis in comparison to pre-dialysis was highly significant [Pvalue <0.0001, table no.1, Graph No 2]. Prabhakar reddy, suchitra M et al [11] have shown intradialytic increase in plasma MDA, decrease in FRAP and uric acid levels. In their study uric acid had significant association with FRAP. The significant decrease in FRAP and uric acid levels in our study in post-dialysis in comparison to predialysis was according to the study done by Prabhakar reddy, suchitra M et al [11] The uremic patients, both dialyzed and nondialyzed, had higher levels of all studied plasma oxidative stress markers and decreased activity of antioxidative enzymes in a study done by kuchta, Agnieszka et al. [12] They had concluded that oxidative stress in their study seem to be related rather to the uremic state than to the dialysis treatment.

N.Nand, H.K Agarwal et al [13] have concluded that hsCRP should be included in the routine laboratory work up for risk elevation in end stage renal disease. Dr. Sumanth kumar B and Dr. B. Shoba rani et al have found elevated hsCRP levels in CKD patients in comparison to control group. [8] The increase in hsCRP mean values in post-dialysis was statistically significant in comparison to pre-dialysis [Pvalue <0.000, table no 1. Graph No. 2]. Ali, Zulkhair et al [14] have concluded that there was significant increase in hsCRP post hemodialysis. However, there was no correlation between dialysis age and hsCRP in their study

The mean values of MDA in our study was decreased significantly during post-dialysis in comparison to pre-dialysis [Pvalue <0.0001, table no.1, Graph No 2).].This decrease may be related to loss of MDA, being a very small molecule, can diffuse across dialytic membranes. Hence a correction of MDA values with creatinine was done and the mean values of MDA micro mol/mg of creatinine was calculated .this was done according to a study conducted by Prabhakar E and suchitra M et al [11].The corrected mean values of MDA per creatinine was increased (p value < 0.0001, table no 1, Graph no 2) during post-dialysis in comparison to pre-dialysis. This was statistically highly significant indicating that there is increase in oxidative stress in post-dialysis.

Hence correcting MDA for creatinine, we found a significant increase in MDA levels indicating that the increase in MDA during HD is due to its increased production, unable to withstand the fact that it is getting cleared by dialysis. This increase in intradialytic MDA levels is an indicator of the presence of oxidative stress during the dialysis session Neetha Kundoor; Shruti Mohanty et al [15] showed significant increase in MDA levels in post-dialysis compared to predialysis in chronic kidney disease patients. De Vecchi, Amedeo F etal16 had shown that MDA concentrations were significantly higher in patients than in controls (ESRD > HD > PD). In their study they had also shown that in PD (Peritoneal dialysis) and HD (Hemodialysis) patients, MDA levels were significantly higher than in ESRD.

A study done by B sangeetha lakshmi, Harinidevi et al [7] had shown increase in inflammatory markers, decrease in FRAP and increase in oxidative stress in the form of MDA in post-dialysis in comparison to predialysis in accordance with our study. However, we could not establish an association of oxidative stress with total antioxidant status in our study.

Conclusion

Chronic kidney disease is a progressive loss in renal function over period of many months or years. Inflammation, oxidative stress and endothelial dysfunction play a very important role in the etiology of chronic kidney disease.

Cardiovascular disease is leading cause of mortality and morbidity in end stage renal disease. Therapeutic interventions likely to reduce oxidative stress can be planned. For example, supplementation of vitamin A and E can be administered to patients on hemodialysis. The decrease in mean values of urea, uric acid and creatinine after dialysis was highly significant in comparison to predialysis samples in our study. There was decrease in FRAP in postdialysis when compared with predialysis and this was statistically highly significant.

Bioincompatible dialysis membranes, impure dialysate, and vascular access are common causes of inflammation during dialysis. In order to reduce

the generation of ROS, bioincompatible dialysis membranes may be replaced by new biocompatible membranes. Further studies can be planned to identify a better dialysate membrane.

Dialysis itself may cause loss of uric acid and other markers leading to decrease in total antioxidant status in the form of FRAP. Uric acid is associated with FRAP as shown in many studies. Use of pure and proper dialysate and appropriate dialytic membrane helps in reducing the oxidative stress and improving antioxidant status. Estimation of parameters is appropriate if proper dialytic membrane is used as it reduces the loss through dialysis.

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