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A STUDY ON THE LEVELS OF MDA AND SOD IN PRE AND POST DIALYSIS PATIENTS WITH CHRONIC KIDNEY DISEASE

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ABSTRACT

The study was conducted on 50 chronic renal failure patients who were undergoing hemodialysis for atleast a period of 6 months. Samples were drawn before and after hemodialysis comprising of two groups A and B. Group C 50 age and gender matched healthy individuals were taken as controls. Serum Malondialdehyde (MDA) when estimated showed significant increase in postdialysis patients when compared with predialysis with $p< 0.0001$. Serum SOD showed a significant decrease in postdialysis patients when compared with predialysis with $p< 0.001$. Prooxidant serum MDA was high in patient groups pre and postdialysis, when compared to controls $p<0.001$, while antioxidant serum SOD was low in patient groups pre and postdialysis when compared to controls $p<0.0001$. In the present study, we observed that patients undergoing hemodialysis are subjected to oxidative stress, due to increase in oxidants and ineffective antioxidant defense mechanisms as shown by decreased serum SOD.

KEYWORDS : Chronic kidney disease, Hemodialysis, Superoxide dismutase, Malondialdehyde

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INTRODUCTION

Chronic kidney disease (CKD) also known as chronic renal disease, is a progressive loss of renal function over a period of months or years. Often CKD is diagnosed as a result of screening of people known to be at risk of kidney problems, such as high blood pressure, diabetes or when it leads to one of its recognized complications such as cardiovascular disease, anemia, or pericarditis[1]. The treatment for CKD can be renal transplantation or dialysis[2]. Dialysis is the mainstay of treatment for patients with End Stage Renal Disease (ESRD) who are waiting for renal transplant or not suitable to undergo renal transplant. It works on the principle of diffusion of solutes and ultrafiltration of fluid across a semipermeable membrane. Adequate dialytic treatment has prolonged the survival of patients with good quality of life. This has unmasked many long term complications of maintenance hemodialysis which are of great concern like cardiovascular disease and atherosclerosis. This may be due to oxidative stress caused by hemodialysis[2]. It has been suggested that dialytic procedure per se may contribute to oxidative stress on account of both leukocyte activation by filter membrane and the outflow of small anti-oxidant hydrophilic solutes. Imbalance between oxidants and antioxidants in the body leads to Oxidative stress, produces free radicals wherein Plasma membrane is the critical target for free radicals[3]. The MDA, a short chain aldehyde is an intermediate product of oxidation of polyunsaturated fatty acids present in plasma membrane and has been used as an indicator of lipid peroxidation reactions[3].

To defend themselves against the free radical attacks, cells have developed various antioxidant systems. Several enzymatic systems like SOD can detoxify free radicals by catalyzing conversion of superoxide anions to hydrogen peroxide and works concomitantly with hydrogen peroxide removing enzymes such as catalase[3]. Oxidative stress has been implicated in a number of pathogenesis associated with uremia[4]. Most of the primary renal diseases resulting in chronic renal failure are inflammatory conditions. As a result of inflammation and progressive decline in glomerular filtration rate (GFR), there is a decrease in clearance of oxidants leading to an imbalance between oxidants and antioxidants. The increase in oxidants with a decrease in antioxidants leads to an amplification of inflammatory response or inactivation of signaling cascade mediating proliferation, differentiation and cell death leading to End Stage Renal Disease. These changes can ultimately alter important structural and functional characteristics and lead to pathological changes[5]. The present study was performed to evaluate alterations in pro-oxidant and antioxidant state in pre and post hemodialysis patients suffering from chronic kidney disease and to compare them with healthy controls. Serum MDA for oxidant state, while serum SOD for antioxidant status of uremic patients undergoing maintenance hemodialysis was analyzed to optimize resources and improve clinical management of these patients.

MATERIALS AND METHODS

It was a population based observational study of 100 subjects, with 50 subjects undergoing hemodialysis for more than 6 months and 50 normal individuals of either sex (male and female) between the age group 30-70 years were included in the study with their consent and approval of ethical committee, carried out in the Department of Biochemistry in association with Department of nephrology (dialysis unit), Kamineni Institute of Medical Sciences, Narketpally, Andhra Pradesh. Patients suffering from Tuberculosis; Malignancy, patients undergoing hemodialysis with acute renal failure and Renal Transplant were excluded from the study. A random venous blood sample (10ml) was collected from controls and from the patients before and after haemodialysis into a sterile disposable syringe which was transferred into centrifuge tubes and was allowed to clot for

30 minutes. The samples were centrifuged at 3000 rotations per minute for 10 minutes and the serum was separated and collected from the centrifuge tubes and stored at -20°C until analysed. Blood Urea was estimated by Berthlot Method[6], Serum Creatinine by Jaffe's Method[7], Serum Malondialdehyde by Thiobarbituric acid reactive substance assay (TBARS) Method[8], serum SOD by inhibition of autooxidation of adrenaline by superoxide dismutase method[9]. Statistical analysis was performed using SPSS software version 11.0. The descriptive results were expressed as mean and standard deviation. Significance of difference between the patient and control groups observed was assessed by using the student 't' test while significance of difference among patients that is before and after haemodialysis was assessed by paired 't' test or wilcoxon signed-rank test if the data were not normally distributed. The p values were expressed along with mean values and standard deviation. The p values less than 0.05 were considered statistically significant. Correlation co-efficient (Pearson's correlation) was calculated to measure the relationship among the variables.

RESULTS

Table No I
Age distribution in patients and controls (N = 50)

AGE GROUPS (YRS)	CONTROLS N = 50	PATIENTS N = 50 (Pre and Post dialysis)
	NUMBER	NUMBER
30-40	7	7
41-50	11	12
51-60	14	21
60- 70	18	10
TOTAL	50	50

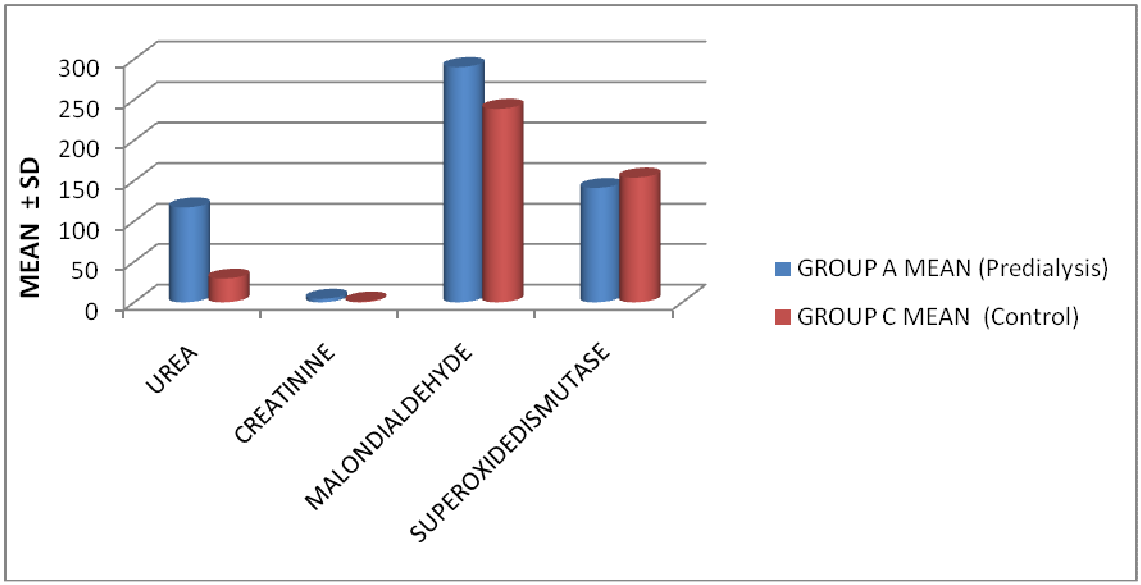
Table I shows the age distribution in patients and controls.

Table II
Biochemical parameters in predialysis patients and controls

PARAMETERS	GROUP A MEAN ± SD N = 50	GROUP C MEAN ± SD N = 50	P – VALUE
UREA (15- 45mg/dl)	117 ± 22.94	29.3 ± 7.46	< 0.0001*
CREATININE (0.7- 1.4 mg/dl)	4.30 ± 2.69	0.97 ± 0.26	<0.0001*
MALONDIALDEHYDE (247 ± 35 mmol/100ml)	290.34 ± 11.34	238.92 ± 17.8	< 0.0001*
SUPEROXIDEDISMUTASE (Activity/ gm protein)	141.22 ± 11.37	153.92 ± 16.19	<0.0001*

Group - A: Pre hemodialysis; Group - C: Controls.
****P was significant if <0. 05***

Graph 1
Biochemical parameters in predialysis patients and controls



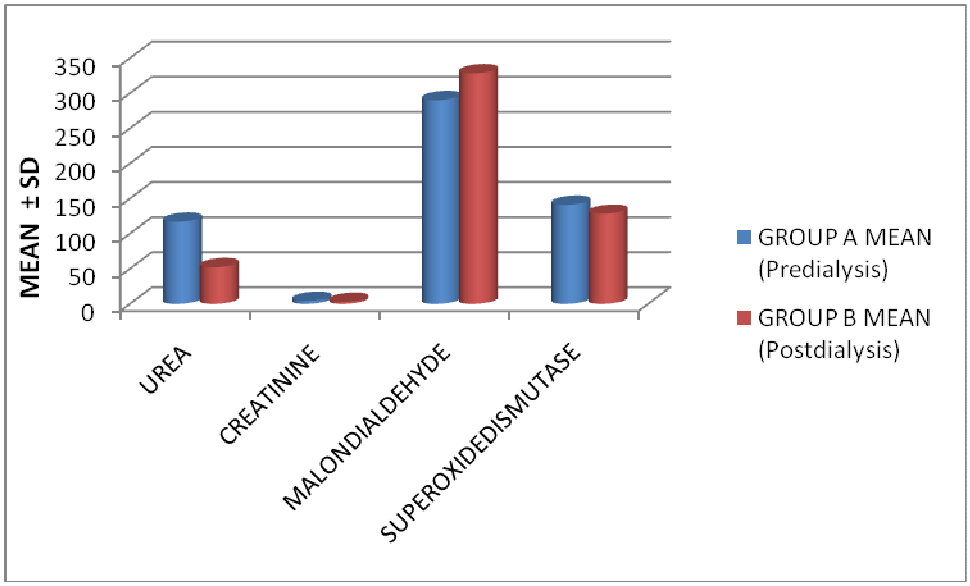
As shown in the table number II (Graph-1)the mean and S.D values of urea and creatinine in pre dialysis patient groups were 117 ± 22.94, 4.30 ± 2.96 respectively and those of controls were 29.3 ± 7.46 and 0.97 ± 0.26 respectively, Serum Urea and Creatinine in predialysis group was significantly high when compared with controls(p<0.0001). The mean and S.D of serum MDA in predialysis patient group were 290.34 ± 11.34, and that of controls were 238.92 ± 17.8, there was a significant increase in MDA levels in predialysis group when compared with controls (p<0.0001).
The mean and S.D of serum SOD in predialysis patients was 141 ± 11.37 and that of controls was 153.92 ± 16.19 shows significant decrease in predialysis patients when compared with controls (p<0.0001).

Table III
Biochemical parameters in pre and postdialysis patients

PARAMETERS	GROUP A MEAN ± SD N = 50	GROUP B MEAN ± SD N = 50	P – VALUE
UREA (15- 45mg/dl)	117 ± 22.94	52.3 ± 19.48	< 0.0001*
CREATININE (0.7- 1.4 mg/dl)	4.30 ± 2.69	2.92 ± 2.25	<0.0001*
MALONDIALDEHYDE (247 ± 35 mmol/100ml)	290.34 ± 11.34	328.52 ± 17.48	< 0.0001*
SUPEROXIDEDISMUTASE (Activity/ gm protein)	141.22 ± 11. 37	128.34 ± 17.50	<0.001*

Group - A: Pre hemodialysis. Group - B: Post hemodialysis.
****P was significant if < 0. 05***

Graph 2
Biochemical parameters in pre and postdialysis patients



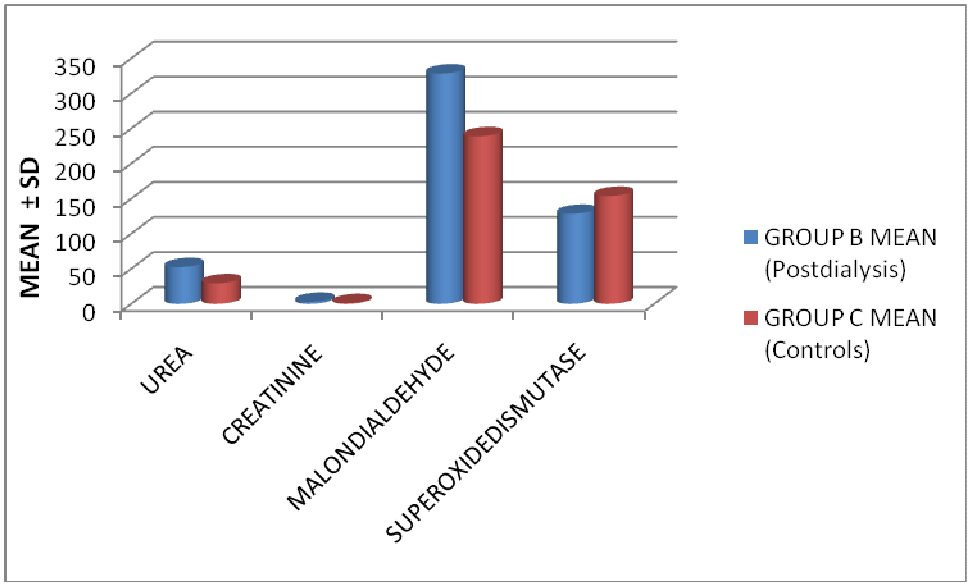
Serum Urea and Creatinine in post dialysis patient group were 52 ± 19.48 and 2.92 ± 2.25 respectively and that in pre dialysis patient group were 117 ± 22.94 and 4.30 ± 2.96 respectively, as shown in the table number III (Graph-2). There was a significant decrease in both the parameters in postdialysis when compared with predialysis groups (p<0.0001) serum MDA is the biomarkers of oxidative stress was measured in pre and post dialysis patient groups. The mean ± S.D in serum MDA in predialysis group was 290.34 ± 11.34 while that of postdialysis group was 328.52 ± 17.48 there was a significant increase in MDA levels in postdialysis when compared with predialysis (p<0.0001). The mean and S.D of serum SOD was significantly high in predialysis group 141.22 ± 11.37 when compared with postdialysis group 128.34 ± 17.50 (p<0.001).

Table IV
Biochemical parameters in postdialysis patients and controls

PARAMETERS	GROUP B MEAN ± SD N = 50	GROUP C MEAN ± SD N = 50	P – VALUE
UREA (15- 45mg/dl)	52.3 ± 19.48	29.3± 7.46	< 0.0001*
CREATININE (0.7- 1.4 mg/dl)	2.92 ± 2.25	0.97±0.26	<0.0001*
MALONDIALDEHYDE (247 ± 35 mmol/100ml)	328.52 ± 17.48	238.92± 17.8	< 0.0001*
SUPEROXIDEDISMUTASE (Activity/ gm protein)	128.34 ± 17.50	153.92 ± 16.19	<0.0001*

Group - B: Post hemodialysis; Group - C: Controls.
****P was significant if <0.05***

Graph 3
Biochemical parameters in postdialysis patients and controls



It was observed that serum Urea and Creatinine in post dialysis group were 52.3 ± 19.48, 2.92 ± 2.25 and that of controls were 29.3 ± 7.46 and 0.97 ± 0.26 respectively, as shown in the table number IV (Graph-3). Both the parameters were significantly high in postdialysis when compared with controls (p<0.0001). Serum MDA showed significant increase 328.52 ± 17.48 in postdialysis patient group when compared with that of the controls 238.92 ± 17.8 (p<0.0001). Serum SOD the biomarker of antioxidant activity was measured in postdialysis patient groups and controls. The mean and S.D of serum SOD in postdialysis was 128.34 ± 17.50 and that of controls 153.92 ± 16.19. Serum SOD showed significant decrease in postdialysis patients when compared with controls (p<0.0001).

DISCUSSION

Oxidative Stress is an imbalance between Oxidants and Antioxidants. The balance between formation of Reactive oxygen species (ROS) and antioxidative defense mechanism depends on the activity of enzymes such as SOD and Catalase. This balance is however fragile, difficult to predict and strongly dependent on environmental conditions. Chronic Kidney Disease (CKD) is characterized by slow and progressive decline in the kidney functions. Some of the manifestations include accelerated aging, cataract, atherosclerosis, increased hemolysis, platelet dysfunction, neuropathy etc. These sequelae of CKD has been attributed to the overproduction of free radicals in these patients[1]. Hemodialysis patients are subjected to Oxidative Stress resulting from the dialysis sessions that is evident due to increased serum concentration of oxidants and decrease in antioxidants. Components of inflammatory response associated with hemodialysis include neutrophil activation, cytokine release, oxidative stress with free radical production, lipid and protein peroxidation and alteration in redox status with blood. The increased concentration of oxidants can be attributed to activation of leukocytes, whether in response to exposure to dialysis membranes or caused by back transport of cytokine-inducing substances from dialysate, increases release of the proinflammatory cytokines, tumor necrosis factor α, and IL-6, which, in turn, increases the expression of cell adhesion molecules on the surface of endothelial cells. Consequently, circulating levels of proinflammtory molecules and cell adhesion molecules may contribute to the increased Oxidative Stress in hemodialysis patients[10]. In this present study variations in oxidants and antioxidant status was observed by estimating serum MDA and SOD levels in

chronic kidney disease patients before and after hemodialysis and compared with the controls .

In our study serum MDA levels showed a statistically significant increase in postdialysis when compared with predialysis and controls .Similar observations were made in another study conducted by Esma Menesve et.al[11]. Another study conducted by Necip Ihlan et[12], observed that the serum MDA levels were significantly decreased in postdialysis when compared with predialysis during hemodialysis with polysulfone membrane while no significant change in the serum MDA levels were observed with the hemophane and cellulose acetate membranes. In the present study, hemodialysis done with cellulose acetate membranes, showed increased neutrophil activation and the rate of formation of oxidants. This could be due to the fact that hemodialysis by the application of a modified circulation and forced passage of blood through a number of filters activates endogenous inflammatory mechanisms and induces chronic release of molecules resulting in an increased production of oxidants. While serum SOD showed a significant decrease in postdialysis when compared with predialysis and further there was statistically significant decrease in serum SOD in postdialysis when compared with predialysis and controls. Study done by Paik-Seong lim et.al[13] showed statistically significant decrease in serum SOD levels in postdialysis patients when compared with predialysis patients. The most probable cause for decreased SOD activity is a possible direct inactivation of the enzyme by its product hydrogen peroxide or by superoxide anion itself. Decreased SOD activity could also be related to trace element deficiencies in hemodialyzed patients and detoxification of superoxide anions is compromised leading to an increased oxidative stress.

CONCLUSION

In the present study it was observed that CKD is more common in more than 60 yrs of age group and most of them were males (76%). Pro-oxidant serum MDA was higher while antioxidant serum SOD was lower in the patient groups (pre and post dialysis) when compared to control group. There was significant increase in serum MDA in postdialysis group when compared to predialysis group and decrease in SOD in Post-dialysis when compared to Pre-dialysis. In accordance with other studies, we also observed that patients undergoing hemodialysis are subjected to oxidative stress, because there is increase in oxidants due to activation of leukocytes by filter membrane, outflow of small antioxidant hydrophilic solutes and ineffective antioxidant defense mechanisms as shown by decreased serum SOD. Further intensive studies have to be conducted to assess and establish the role of different types of dialysis membranes on oxidative stress in patients undergoing hemodialysis.

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