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IMPACT OF SINGLE SESSION OF DIALYSIS ON OXIDATIVE STRESS AND INFLAMMATORY MARKERS ON CHRONIC KIDNEY DISEASE

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ABSTRACT

Background: Chronic kidney disease or CKD has a high prevalence globally with associated high mortality and morbidity rates posing a significant health concern. With its progression, oxidative stress and inflammation are seen in affected subjects. The most common RRT (renal replacement therapy) is hemodialysis in Indian subjects.

Aim: The present study aimed to comparatively assess the biochemical profile, oxidative markers, and immune modality profiles in pre and post-dialysis subjects with chronic kidney disease.

Methods: The present study assessed 50 subjects with chronic kidney disease visiting the Institute and with a mean age of 60±12 years. The included subjects were undergoing dialysis three times a week for 4 hours of every session at the flow rate of 250-300 mL/min. Oxidative stress markers and inflammatory markers were assessed in all the subjects before and after dialysis in any one of the dialysis sessions of the included subjects.

Results: An improvement in kidney function was seen following dialysis in CKD subjects along with a significant decrease in the oxidative stress and inflammatory markers following the single session of dialysis. Also, a significant reduction in other biochemical markers of serum was seen in study subjects after dialysis.

Conclusions; The present study concludes that improvement in renal function along with a decrease in oxidative stress and inflammatory markers is seen following hemodialysis in subjects with chronic kidney disease. The study also suggested that hemodialysis is a better and most common treatment option in subjects with chronic kidney disease.

Keywords: chronic kidney disease, dialysis, inflammatory markers, oxidative stress, renal function

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INTRODUCTION

Chronic kidney disease or CKD has a high prevalence globally with associated high mortality and morbidity rates posing a significant health concern. Following the data of 2013 from the Global Burden of Disease study, it was reported that nearly 9.56,200 subjects died from chronic kidney disease reporting a 13% increase from the data from 1990. This increase since 1990 was one of the most significant increases among the top reasons for mortality. The number of deaths reported from chronic kidney disease in India showed a significant increase from 0.5 million in 1990 data to 1.18 million in the year 2016. It is considered that the singular increasing trend in the mortality and prevalence of chronic kidney disease mortality should be taken as a global public health priority.[1]

Chronic kidney disease is a significant concern globally about 1.4 million subjects globally having ESRD (end-stage renal disease) are estimated to undergo RRT (renal replacement therapy) with transplantation or dialysis with a reported annual growth of 8%. Owing to the challenges in the accessibility to health care in developing countries such as India, more than 50% of the subjects having advanced chronic kidney disease report to healthcare with an eGFR of <15 mL/min per 1.73 m2. A sharp increase in the cases of chronic diseases including diabetes and hypertension poses a risk factor for chronic kidney disease which also helps in increasing the prevalence of chronic kidney disease posing a high burden on the healthcare sector.[2]

CKD or chronic kidney disease is defined as an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m2 or the presence of kidney damage that persists for three months or more with non-consideration of the disease cause. CKD also presents the state of progressive kidney function loss which ultimately results in the need for RRT as either transplantation or dialysis. High levels of metabolic end-products such as uremic toxins have a potential clinical role in the progression of chronic kidney disease and are closely associated with various complications of chronic kidney disease.[3]

Subjects with chronic kidney disease suffer from related various complications including metabolic acidosis, anemia, cardiovascular diseases, neurological complications, bone and mineral disturbances, altered immune responses, and hypertension. Among these complications, infections secondary to disturbed and altered immune systems and cardiovascular dysfunctions may lead to increased risk of mortality and morbidity in these subjects. In such conditions, oxidative stress and inflammation play a vital role. With the progression of chronic kidney disease, an increase in oxidative stress and inflammatory markers is seen.[4]

Subjects with chronic kidney disease usually have chronic inflammation along with severely impaired anti-oxidative systems which progressively worsen with the renal failure degree. Oxidative stress and inflammation are vital in the defense mechanisms against various infections, however, when they are not regulated properly, they can initiate various deleterious effects including the increase in the mediators of oxidative stress and inflammation along with cytokine overproduction.[5]

Oxidative stress is generally seen in subjects with ESRD or CKD and is a non-traditional risk factor for all the mortality cases in these subjects. Hence, treatment of oxidative stress and

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inflammation in CKD subjects is primarily vital in the uremic syndrome cases. The most common RRT (renal replacement therapy) in CKD subjects is hemodialysis in the Indian context. The data from 2018 estimates that for several subjects undergoing chronic hemodialysis in India is 129 subjects per million subjects.[6]

The estimated number of hemodialysis stations in India is considered to be 12.881 following the data of 2018. However, despite hemodialysis being the treatment of choice in renal replacement therapy, the risk associated with CKD concerning the upregulation of oxidative stress and inflammation has not been studied extensively. Limited literature data with very few studies concerning the effects of acute hemodialysis sessions on oxidative stress and inflammation in CKD subjects is documented.[7]

The present study aimed to comparatively assess the biochemical profile, oxidative markers, and immune modality profiles in pre and post-dialysis subjects with chronic kidney disease.

MATERIALS AND METHODS

The present cross-sectional study aimed to comparatively assess the biochemical profile, oxidative markers, and immune modality profiles in pre and post-dialysis subjects with chronic kidney disease. The study was done at Department of Medicine, Saraswathi Institute of Medical Sciences, Hapur, Uttar Pradesh after the clearance was given by the concerned Institutional Ethical committee. The study subjects were recruited after obtaining verbal and written informed consent from all the participants.

The study included 50 subjects from both genders having ESRD (end-stage renal disease) and was from the Department of Nephrology of the Institute along with 50 age and gender-matched controls. All the included study participants were given regular hemodialysis for three times a week for 4 to 5 hours for each session via arteriovenous fistulas.

In the study, 50 healthy subjects comprising relatives of the staff of the institute, non-diabetics, and non-smokers following the criteria of ADA (American diabetic association), and were non-hypertensive following JNC (Joint National Committee) VIII comprised the control group. The exclusion criteria for the study were subjects not willing to participate in the study, pregnant females, infective diseases, chronic inflammation such as liver disease or malignancy, hepatitis B/C infection, HIV, age less than 18 years, and on hemodialysis for less than 3 months.

In the maintenance hemodialysis program, low-flux conventional intermittent hemodialysis was done thrice weekly as maintenance RRT (renal replacement therapy) using bicarbonate dialysate, polysulfone membrane, and hemodialysis machine with a blood flow rate of 200-300 mL/min and dialysate flow of 500 mL/min for 4 hours duration. Reverse osmosis was used for the purification of water for dialysate. 2000 IU heparin was used for anticoagulation at initiation of dialysis followed by continuous supply at 500-1000 IU/hour rate. Pre-dialysis sample was taken from the afferent fistula needle before the start of dialysis before its connection with the dialyzer and the post-dialysis sample after 4 hours from the same port before terminating the dialysis procedure.

Just before starting the dialysis and at dialysis termination, 6 ml of venous blood was taken for laboratory tests in test tubes without anticoagulant. The blood samples were centrifuged at 3000 rpm for 15 minutes after standing for 30 minutes and serum separated was collected and stored at

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-80^{o}C before analyzing. Commercial kits were used to assess albumin, total protein, phosphorus, calcium, HDL, triglycerides, total cholesterol, uric acid, creatinine, and urea in the serum. Freidewald's equation[8] was used to calculate VLDL (very-low-density lipoprotein) and LDL (low-density lipoprotein). Hs-CRP was assessed using the immunoturbidimetry method and a commercially available kit. All the parameters were assessed in the laboratory with ELISA being used to assess the Pentraxin-3 and plasma MDA and the ferric-reducing ability of plasma was assessed with a spectrophotometer.

Serum hs-CRP and serum Pentraxin samples post-dialysis were corrected using ultrafiltration utilizing the formula as $1 + \Delta BW/0.2 \times BW$[9] where BW denotes a change in the body weight during hemodialysis and extracellular volume was 20% of post-dialysis BW considering uniform ultrafiltration during hemodialysis. Serum MDA was corrected to serum creatinine and was expressed in µmol/mg of creatinine.

The data gathered were analyzed statistically using the SPSS software version 21.0 (IBM Corp., Armonk, NY, USA) with the Mann-Whitney U-test and chi-square test. The data were expressed as mean and standard deviation and frequency and percentage. Statistical significance was kept at a p-value of <0.05. To evaluate the change in parameters of any group before and after surgery, repeated measurements and ANOVA (analysis of variance) were used.

RESULTS

The present cross-sectional study aimed to comparatively assess the biochemical profile, oxidative markers, and immune modality profiles in pre and post-dialysis subjects with chronic kidney disease. The present study assessed 50 subjects with chronic kidney disease visiting the Institute and with a mean age of 60±12 years. The mean age of subjects in the control and hemodialysis group was 50.04±6.24 and 50.02±5.88 years respectively showing non-significant with p=0.511. The mean BMI was 24.02 (18-28.08) and 23.02 (19-26.06) kg/m2 which was statistically non-significant with p=0.227. There were 44% (n=22) females and 56% (n=280 males in both the control and hemodialysis groups as shown in Table 1.

On comparison of routine biochemical parameters at baseline in two groups of study subjects, Significantly higher values of uric acid, phosphorus, cholesterol, creatinine, and serum urea were seen in kidney disease subjects compared to controls, and these values decreased significantly following dialysis with p-values of <0.01, 0.01, 0.02, <0.01, and <0.01 respectively. A significantly lower value of calcium, sodium, and LDL was seen in kidney disease subjects compared to the controls which significantly increased following hemodialysis with p-values of <0.01, <0.01, and <0.01 respectively. A non-significant difference was seen for VLDL, HDL, triglycerides, total protein, and albumin in controls and hemodialysis subjects with p=0.612, 0.06, 0.341, 0.314, and 0.28 respectively as depicted in Table 2.

Concerning the comparison of biochemical parameters in the pre-dialysis and control group, uric acid, phosphorus, potassium, sodium, cholesterol, creatinine, and urea were significantly higher in the hemodialysis group compared to the controls with p-values of <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, and <0.1 respectively. LDL levels were significantly lower in a pre-dialysis group compared to controls with p<0.01. A non-significant difference was seen for calcium, VLDL,

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HDL, triglycerides, total proteins, and albumin with p=0.100, 0.310, 0.814, 0.345, 0.204, and 0.83 respectively (Table 3).

On comparison of pre-dialysis and post-dialysis biochemical parameters in study subjects, a significant decrease in uric acid, phosphorus, potassium, creatinine, and urea levels was seen with p<0.01, <0.01, <0.01, <0.01, and <0.01 respectively. Also, a significant increase was seen in calcium, sodium, and LDL levels with p<0.01, 0.01, and 0.01 respectively. However, a non-significant difference was seen for VLDL, HDL, cholesterol, triglycerides, total protein, and albumin levels with p-values of 0.541, 0.181, 0.08, 0.266, 0.87, and 1.44 respectively as summarized in Table 4.

For the comparison of special biochemical parameters at baseline in various study groups, FRAP (ferric reducing ability of plasma) was higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p=0.01. Malondialdehyde (MDA) in serum was significantly higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p<0.001. hs-CRP (high sensitivity C-reactive protein) was significantly higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p<0.001. Pentraxin was significantly higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p<0.001 as shown in Table 5.

On comparing the biochemical parameters of hemodialysis in pre and post-dialysis groups at baseline, a significant increase in MDA, hs-CRP, and pentraxin levels was seen post-dialysis compared to pre-dialysis with p-values of <0.001, <0.001, and <0.001 respectively. FRPA values were significantly higher pre-dialysis and decreased significantly pot-dialysis with p=0.03 as shown in Table 6.

DISCUSSION

The present study included 50 subjects with chronic kidney disease visiting the Institute and with a mean age of 60±12 years. The mean age of subjects in the control and hemodialysis group was 50.04±6.24 and 50.02±5.88 years respectively showing non-significant with p=0.511. The mean BMI was 24.02 (18-28.08) and 23.02 (19-26.06) kg/m2 which was statistically non-significant with p=0.227. There were 44% (n=22) females and 56% (n=280 males in both the control and hemodialysis group. These results were similar to the studies of Gerardi G et al[10] in 2002 and Reddy PE et al[11] in 2011 where authors assessed subjects with chronic kidney disease and demographic data comparable to the present study.

It was seen that on comparison of routine biochemical parameters at baseline in two groups of study subjects. Significantly higher values of uric acid, phosphorus, cholesterol, creatinine, and serum urea were seen in kidney disease subjects compared to controls, and these values decreased significantly following dialysis with p-values of <0.01, 0.01, 0.02, <0.01, and <0.01 respectively. A significantly lower value of calcium, sodium, and LDL was seen in kidney disease subjects compared to the controls which significantly increased following hemodialysis with p-values of <0.01, <0.01, and <0.01 respectively. A non-significant difference was seen for VLDL, HDL, triglycerides, total protein, and albumin in controls and hemodialysis subjects with p=0.612, 0.06, 0.341, 0.314, and 0.28 respectively. These results were consistent with the findings of Ansarihadipour H et al[12] in 2014 and Reddy PE et al[13] in 2010 where authors reported significantly

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higher values of uric acid, phosphorus, cholesterol, creatinine, and serum urea were seen in kidney disease subjects compared to controls s seen in the results of the present study.

The study results showed that for the comparison of biochemical parameters in the pre-dialysis and control group, uric acid, phosphorus, potassium, sodium, cholesterol, creatinine, and urea were significantly higher in the hemodialysis group compared to the controls with p-values of <0.01, <0.01, <0.01, <0.01, <0.01, <0.01, and <0.1 respectively. LDL levels were significantly lower in a pre-dialysis group compared to controls with p<0.01. A non-significant difference was seen for calcium, VLDL, HDL, triglycerides, total proteins, and albumin with p=0.100, 0.310, 0.814, 0.345, 0.204, and 0.83 respectively. These findings were in agreement with the results of Ramakrishna P et al[14] in 2012 and Hacisevki A et al[15] in 2008 here authors, similar to the present study, reported uric acid, phosphorus, potassium, sodium, cholesterol, creatinine, and urea as significantly higher in hemodialysis group compared to the controls

Concerning the comparison of pre-dialysis and post-dialysis biochemical parameters in study subjects, a significant decrease in uric acid, phosphorus, potassium, creatinine, and urea levels was seen with p<0.01, <0.01, <0.01, <0.01, and <0.01 respectively. Also, a significant increase was seen in calcium, sodium, and LDL levels with p<0.01, 0.01, and 0.01 respectively. However, a non-significant difference was seen for VLDL, HDL, cholesterol, triglycerides, total protein, and albumin levels with p-values of 0.541, 0.181, 0.08, 0.266, 0.87, and 1.44 respectively. These results were in line with Matsubara K et al[16] in 2009 and Malaponte G et al[17] in 2007 where authors suggested a significant decrease in uric acid, phosphorus, potassium, creatinine, and urea levels post-dialysis in chronic kidney subjects.

Concerning the comparison of special biochemical parameters at baseline in various study groups, FRAP (ferric reducing ability of plasma) was higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p=0.01. Malondialdehyde (MDA) in serum was significantly higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p<0.001. hs-CRP (high sensitivity C-reactive protein) was significantly higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p<0.001. Pentraxin was significantly higher in a pre-dialysis group compared to controls that reduced significantly post-dialysis with p<0.001. These results were comparable to the studies of Tong M et al[18] in 2007 and Korevaar JC et al[19] in 2004 where authors reported FRAP, MDA, hs-CRP, and pentraxin as significantly higher in pre-dialysis subjects compared to controls.

It was seen that on comparing the biochemical parameters of hemodialysis in pre and post-dialysis groups at baseline, a significant increase in MDA, hs-CRP, and pentraxin levels was seen post-dialysis compared to pre-dialysis with p-values of <0.001, <0.001, and <0.001 respectively. FRPA values were significantly higher pre-dialysis and decreased significantly pot-dialysis with p=0.03. These results were similar to Peri G et al[20] in 2000 and Boheme M et al[21] in 2007 where authors reported a significant increase in MDA, hs-CRP, and pentraxin levels post-dialysis compared to pre-dialysis subjects.

CONCLUSIONS

Considering its limitations, the present study concludes that improvement in renal function along with a decrease in oxidative stress and inflammatory markers is seen following hemodialysis in

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subjects with chronic kidney disease. The study also suggested that hemodialysis is a better and most common treatment option in subjects with chronic kidney disease. However, further longitudinal studies are required to reach definitive conclusions.

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TABLES

Characteristics	Controls (n=50)	Hemodialysis (n=50)	p-value
BMI (kg/m2)	24.02 (18-28.08)	23.02 (19-26.06)	0.227
Mean age (years)	50.04±6.24	50.02±5.88	0.511
Gender n (%)			
Females	22 (44)	22 (44)	1.000
Males	28 (56)	28 (56)	

Table 1: Comparison of demographic data in two groups of study subjects

Parameters	Pre-dialysis	Post-dialysis	Controls	p-value
Uric acid (mg/dl)	5.12 (2.92-8.78)	2.12 (1.22-5.44)	4.02 (2.32-5.58)	**<0.01**
Phosphorus (mg/dl)	4.50 (2.82-12.24)	3.02 (1.32-7.44)	3.7 (2.7-4.4)	**0.01**
Calcium (mg/dl)	8.8 (7.32-10.40)	9.7 (7.9-10.8)	9.1 (8.3-9.7)	**<0.01**
Potassium (mmol/L)	4.42 (3.22-5.58)	2.88 (2.22-4.78)	3.6 (3.3-4.3)	**<0.01**
Sodium (mmol/L)	128 (120-131)	129 (121-133)	137 (128-141)	**<0.01**
VLDL (mg/dl)	25 (8-55)	24 (8-55)	26 (16-52)	0.612
LDL (mg/dl)	70 (31-137)	87 (40-156)	108 (84-148)	**<0.01**
HDL (mg/dl)	40 (24-48)	41 (27-58)	40 (29-51)	0.0666
Cholesterol (mg/dl)	175 (153-220)	154 (94-232)	144 (86-214)	**0.02**

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Triglycerides (mg/dl)	124 (58-283)	118 (49-285)	131 (88-266)	0.341
Total proteins (g/dl)	7.40 (5.92-9.34)	7.72 (5.74-9.72)	(6.8-8.6)	0.314
Albumin (g/dl)	3.92 (3.02-4.44)	4.02 (2.92—4.76)	4.3 (3.2-4.9)	0.28
Creatinine (mg/dl)	7.54 (4.24-13.27)	3.76 (1.57-11.52)	0.84 (0.56-1.62)	**<0.01**
Urea (mg/dl)	96 (44-145)	37 (23-76)	20 (16-48)	**<0.01**

Table 2: Routine biochemical parameters at baseline in two groups of study subjects

Parameters	Pre-dialysis (Mean rank)	Controls (Mean rank)	p-value
Uric acid (mg/dl)	33.32	17.64	**<0.01**
Phosphorus (mg/dl)	32.78	18.22	**<0.01**
Calcium (mg/dl)	22.14	28.86	0.100
Potassium (mmol/L)	33.42	17.62	**<0.01**
Sodium (mmol/L)	13.56	37.44	**<0.01**
VLDL (mg/dl)	23.44	27.56	0.310
LDL (mg/dl)	15.88	35.12	**<0.01**
HDL (mg/dl)	25.04	25.96	0.814
Cholesterol (mg/dl)	33.42	17.58	**<0.01**
Triglycerides (mg/dl)	23.62	27.34	0.345
Total proteins (g/dl)	22.88	28.12	0.204
Albumin (g/dl)	21.94	29.02	0.83
Creatinine (mg/dl)	37.98	13.02	**<0.01**
Urea (mg/dl)	37.94	13.06	**<0.01**

Table 3: Comparison of biochemical parameters in pre-dialysis and control group

Parameters	Pre-dialysis (Mean rank)	Post-dialysis (Mean rank)	p-value
Uric acid (mg/dl)	5.12 (2.88-8.78)	2.12 (1.18-5.38)	**<0.01**
Phosphorus (mg/dl)	4.50 (2.78-12.18)	3 (1.28-7.42)	**<0.01**
Calcium (mg/dl)	8.8 (7.28-10.28)	9.68 (7.7-10.6)	**<0.01**
Potassium (mmol/L)	4.42 (3.4-5.4)	2.88 (2.18-4.78)	**<0.01**
Sodium (mmol/L)	126 (120-131)	129 (121-133)	**0.01**
VLDL (mg/dl)	25 (8-55)	24 (8-55)	0.541
LDL (mg/dl)	70 (31-137)	87 (40-156)	**0.01**
HDL (mg/dl)	40 (24-48)	39 (27-58)	0.181
Cholesterol (mg/dl)	175 (153-220)	154 (94-232)	0.08
Triglycerides (mg/dl)	124 (58-283)	118 (49-285)	0.266
Total proteins (g/dl)	7.42 (5.88-9.32)	7.68 (5.72-9.68)	0.87
Albumin (g/dl)	3.92 (3.02-4.44)	4 (2.92-4.70)	1.44
Creatinine (mg/dl)	7.54 (4.24-13.23)	3.76 (1.57-11.52)	**<0.01**
Urea (mg/dl)	96 (44-145)	39 (19-76)	**<0.01**

Table 4: Comparison of biochemical parameters in pre and post-dialysis group

Parameters	Pre-dialysis	Post-dialysis	Controls	p-value
FRAP (mmol/L)	0.82 (0.53-1.16)	0.42 (0.23-0.84)	0.55 (0.43-0.97)	**0.01**
MDA (µmol/L)	3.96 (2.31-6.43)	3.35 (2.12-6.22)	1.22 (0.53-1.89)	**<0.001**
Hs-CRP (mg/dl)	1.82 (1.12-5.78)	1.83 (1.14-5.88)	0.31 (0.23-1.02)	**<0.001**
Pentraxin (ng/mL)	1.94 (1.64-2.74)	1.73 (1.42-2.32)	0.36 (0.22-0.63)	**<0.001**

Table 5: Biochemical parameters of hemodialysis in various study groups at baseline

Parameters	Pre-dialysis	Post-dialysis	p-value

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FRAP (mmol/L)	0.82 (0.53-1.16)	0.71 (0.34-1.17)	**0.03**
MDA (µmol/L)	3.73 (2.13-6.16)	3.74 (2.29-6.55)	**<0.001**
Hs-CRP (mg/dl)	1.82 (1.12-5.78)	2.16 (1.42-6.15)	**<0.001**
Pentraxin (ng/mL)	1.94 (1.64-2.74)	2.03 (1.69-2.61)	**<0.001**

Table 6: Biochemical parameters of hemodialysis in pre and post-dialysis groups at baseline