Original article:

Evaluation of oxidative stress and antioxidant activity in pre and post hemodialysis in chronic renal failure patients from Western region of Nepal

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Abstract:

Objective: To evaluate the oxidative stress and antioxidant activity in pre and post hemodialysis in chronic renal failure patients using a diacetate cellulose membrane.

Background: Chronic Renal Failure is a gradual, progressive and irreversible loss of normal functioning of kidneys. This is associated with increased formation of reactive oxygen species and increased oxidative stress. No studies have been undertaken in this area in Nepal.

Method: The study groups were devided into chronic renal failure patients undergoing hemodialysis (n=52) and healthy controls (n=52). Diacetate cellulose was used as dialyzer membrane for chronic renal failure patients. Blood samples were collected in EDTA bottles and plasma was used for the estimation of oxidative stress marker, total antioxidant activity, vitamin C, α-tocopherol, urea and creatinine.

Results: There was significant reduction in the levels of vitamin C,α -tocopherol and total antiox- idant activity in post dialysis patients compared to predialysis and control groups ($p<0.001$). Plasma urea and creatinine levels were high in predialysis patients compared to controls and was significantly reduced after dialysis. Significantly elevated malondialdehyde levels were found in chronic renal failure patients before and after dialysis when compared with controls.

Conclusion: The results of our study indicate that there are reduced levels antioxidants in chronic renal patients in pre and post dialysis condition and also more loss of antioxidants after dialysis. All these condition leads to increased oxidative stress in these patients.

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Introduction:

Chronic renal failure (CRF) is a pleuricausal disease due to progressive and irreversible attrition of nephrons regardless of the setting cause and considered to exist when glomerular filtration rate (GFR) is reduced for at least 3-6 months. Frequently progressive decline is seen over a period of years. The global incidence of end stage renal disease is 242 per million, which in all probability is an underestimate and the incidence may rise sharply in recent future due to rapidly rising uncontrollable pollution and lifestyle changes[1]. In Nepal the overall prevalence of CRF in urban areas is 10.6%[2]. In CRF patients, heightened oxidative stress is due to increased generation of reactive oxygen species (ROS). Increase in ROS is due to exposure of blood to dialysis membrane, uremic toxicity and less intake of dietary antioxidants. Weakened status of antioxidants could be a discerning cause in many CRF and other renal disease patients[3-5].

The nettled oxidative stress is believed to accentuate the ischemic, toxic and immunologically mediated renal tubular and glomerular cell injury. Experimental studies lend considerable support to this hypothesis, which demonstrate that ROS has exacerbating interaction with different mediators of oxidative stress such as eicosanoids, platelet activating factor, reactive nitrogen species and proteases[6-8].

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Malondialdehyde (MDA) is the oxidative end product of polyunsaturated fatty acids and the increased concentration of MDA has been reported in hemodialysed patients[4]. The primary defense against the oxidative stress is played by nutritional antioxidants and endogenous antioxidants. Some reports have shown controversial differences in plasma MDA and non-enzymatic antioxidants between hemodialysis patients and controls[9-10].

The aim of this study included to evaluate the effect of oxidative stress, antioxidant activity in pre and post hemodialysis in chronic renal failure patients by using diacetate cellulose membrane.

Materials and Methods

The study was approved by the ethical committee of the Manipal College of Medical sciences, Nepal. Prior verbal consent was taken from all the participants. The study included a total of 52 patients (mean age=64±7yrs) diagnosed with chronic renal failure from Nephrology department, Manipal teaching hospital, Pokhara, Nepal. All patients who underwent this first dialysis were selected for evaluating the effect of hemodialysis. Diacetate cellulose was used as dialyzer membrane. Normal subjects (n = 52, mean age= 57±12yrs) without any disease and not taking any drug/ medication that would affect the oxidant and antioxidant level were included in the study. Ten ml blood was collected in EDTA bottles from anticubital vein before and after hemodialysis. Samples were centrifuged at 3000 rpm for 10 minutes. Plasma was transferred to another labeled vial for immediate analysis or stored at -20^{o}C. The plasma was used for the estimation of malondialdehyde (MDA), [11] total antioxidant activity (TAA) [12], vitamin C[13], α-tocopherol[14] by standard methods. Urea and creatinine were estimated by Ranbaxy kit method.

Statistical analysis

Results are expressed as mean±SD. Statistical analysis was done using SPSS 16 version. Significance was tested using independent sample t-test and relationships between the variables were evaluated with the Pearson's correlation coefficient (r). $p<0.05$ was considered as statistically significant.

Results

Table 1 shows that there was significant evidence of lipid peroxidation in pre and post dialysis patients as compared to the control group. Vitamin C, α-toco- pherol and TAA were significantly reduced in post dialysis group when compared with pre dialysis and control group ($p<0.001$). Plasma urea and creatinine were significantly increased when compared with control group and significantly reduced after dialysis when compared with predialysis patients. No signif- icant change in MDA level was observed in post dialysis patients when compared to pre dialysis patients.

Table 2 and 3 shows the correlation coefficient of MDA with other parameters. MDA was found to be weakly correlated with vitamin C and TAA in predialysis and post dialysis patients. However none of the correlations were found significant.

Discussion:

Kidneys being exceptionally rich in vascular tissues, blood supply and metabolically very active organ, are thought to be one of the most susceptible organ to ROS injury. The oxidative stress (OS) was considerably raised in CRF patients before and after hemodialysis when compared to the control group. Raised OS of different magnitude in CRF patients has been reported by different workers. For example chung et al observed three times raised OS (9.40 nmol/l) in CRF patients[15]. Likewise various observations have been reported significantly raised level of MDA in pre and post dialysis patients when compared with controls[16-18]. Our observations indicate that raised OS in CRF patients is due to imbalance between free radicals, ROS and antioxidant scavenging system. It also indicates that dialyzer membrane is not playing any role in reducing oxidative stress. The use of vitamin E coated cellulose membrane will help to decrease the oxidative stress[19-20].

To counterbalance the OS with in physiological limits the body tissues have an ingenious intricately woven network of antioxidants, in which dietary antioxidants play first line of strong defense. There is accumulating evidence that shows the measurement of vitamin C, α-tocopherol and TAA in serum or plasma relays for better information about ROS scavenging strength[1,21]. In our study we observed that α-tocopherol and TAA were significantly decreased in pre and post dialysis patients when compared with controls. Our observations indicate that most of the dietray antioxidants are utilized to reduce the raised oxidative stress and also due to impaired absorption of dietary nutrients because of

great disturbance in lipid metabolism[22-24]. Statistically significant change was observed in the levels of vitamin C, α-tocopherol, TAA, urea and creatinine of post dialysis patients when compared with controls. Vitamin C level was significantly raised in pre dialysis patients when compared with controls and more decreased in post dialysis patients. Decreased vitamin C concentration in post dialysis is due to removal of antioxidants along with other waste metabolites because of its water soluble nature[25-28] and increased concentration in pre dialysis is may be to maintain α- tocopherol in its non-radi- cal reduced form or not being utilized by the defense mechanism.

Hemodialysis is not supposed to be an active participant in prooxidant or antioxidant activity as its role is to remove the waste metabolites from blood. As such, its beneficial role in this process would be only to remove the oxidative adducts and retain the effective antioxidants. So far, to the best of our knowledge, except vitamin E coated membrane no dialysis membranes are specifically designed keeping this particular aspect in consideration. But in our study the TAA levels were significantly reduced after dialysis without any significant change in MDA levels. This may be due to elimination of most of the antioxidant during hemodialysis.

In conclusion, our data collectively suggest that changes in OS and antioxidant status in CRF patients are more likely due to defective kidney functioning and this shows that oxidative stress is accompanied by decreased antioxidant defense in CRF patients on hemodialysis. This problem can be overcome by using advanced dialysis membrane and by giving exogenous supplementation of antioxidants to improve the health status of CRF patients. We strongly believe that our study results serve as preliminary data to plan such studies in future in Nepali population.

Table:1 Oxidative stress and antioxidant status in controls and cases

	Controls	Pre dialysis	Post dialysis
MDA (nmol/ml)	1.3±0.75	4.3±1.4*	4.5±2.1*
Vitamin C (mg/dl)	0.84±0.32	1±0.31**	0.54±0.18*
α - tocopherol (mg/dl)	0.7±0.42	0.43±0.1*	0.25±0.1*
TAA (µmol/L)	835±193	513±147*	386±194*
Urea (mg/dl)	30.8±6.1	236±36*	123±39*
Creatinine (mg/dl)	1±0.32	11±1.7*	6.06±2.3*

*p is <0.001 in predialysis and postdialysis Vs controls

**p = 0.009 in predialysis Vs controls

Table: 2 Pearson's correlation coefficient (r) between malondialdehyde and antioxidant parameters among pre dialysis patients

Parametres	r	P
MDA Vs Vitamin C	0.107	0.449
MDA Vs α-tocopherol	-0.027	0.850
MDA Vs TAA	-0.313	0.24
MDA Vs Urea	-0.029	0.840
MDA Vs Creatinine	0.158	0.264

Table: 3 Pearson's correlation coefficient (r) between malondialdehyde and antioxidant parameters among post dialysis patients

Parametres	r	P
MDA Vs Vitamin C	-0.024	0.864
MDA Vs α-tocopherol	0.019	0.894
MDA Vs TAA	0.179	0.204
MDA Vs Urea	0.133	0.349
MDA Vs Creatinine	-0.099	0.485

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