Research Article

Evaluation of Oxidative Stress Indices in Pre and Post Hemodialysis Cases of Chronic Renal Failure Patients

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ABSTRACT

Objective: This study was undertaken to evaluate the effect of oxidative stress in chronic renal failure patients before and after hemodialysis and compared to healthy control individuals. Materials & Methods: The study comprised of 30 hemodialysis patients compared with 30 healthy controls. The object of the present study was to investigate the possible free radical mediated tissue damage in CRF before and after dialysis, by measuring Melondialdehyde (MDA) which is marker of oxidative stress and L-carnitine for lipid peroxidation. Results: MDA levels were found to be significantly increased in pre dialysis CRF patients as compared to the controls ($P < 0.001$) and the levels further increased in post dialysis CRF patients as compared to that in pre dialysis CRF patients ($P < 0.001$). Antioxidant enzymes such as superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase and the levels of antioxidants like Vitamin A, Vitamin E and Vitamin C along with L-carnitine were significantly decreased in pre dialysis patients as compared to the controls ($P<0.001$) and the values further significantly decreased in post dialysis patients as compared to the pre dialysis CRF patients ($P<0.001$). Conclusion: Our study have shown there is considerable oxidative stress in patients with CRF, which is further exacerbated by hemodialysis, as evidenced by increased lipid peroxidation and low levels of antioxidant and L-carnitine.

Keywords: Hemodialysis, L-carnitine, melondialdehyde, oxidative stress

Received 18 Oct 2012 Received in revised form 26 Oct 2012 Accepted 29 Oct 2012

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INTRODUCTION:

Chronic renal failure is a syndrome characterized by progressive and irreversible deterioration of renal function due to slow destruction of renal parenchyma, eventually terminating in death when sufficient numbers of nephrons have been damaged. Acidosis is the major problem in CRF with development of biochemical azotemia and clinical uraemia syndrome [1]. In recent years Hemodialysis has been successful in extending life span of renal patients and is effective in correcting the metabolic abnormalities related to renal oxidative stress that contributes to morbidity in Hemodialysis patients [2]. Oxidative stress, by definition, is a biochemical condition in which oxidant species overwhelm antioxidant defense ultimately leading to a given biological damage [3, 4].

L-carnitine is an essential factor for the membrane transport of acyl-CoA compounds, particularly for the intramitochondrial transport of long-chain fatty acids [5]. L-carnitine also helps to remove by-products of fatty acid metabolism and other toxic compounds from the cells [6]. The liver and kidney represent the main sources of endogenous carnitine synthesis [7]. Also among the homeostatic processes controlling the endogenous L-carnitine pool in humans, the kidney has a vital role through extensive and adaptive tubular reabsorbtion [8]. Kidney disease can lead to disturbances in L-carnitine homeostasis, and carnitine deficiency may occur in hemodialysis patients leads to oxidation of fatty acids and lipid metabolisms are

severely affected. Bellinghieri et al reported that hemodialysis may promote excessive losses of L-carnitine [9]. Aberrant fatty acid metabolism has been associated with the pro-motion of free radical production, insulin resistance and cellular apoptosis [10]. Oxidative stress is assessed by measuring malondialdehyde (MDA), which is a marker and a product of lipid peroxidation. The study also included the evaluation of antioxidant enzymes, L-carnitine, Non-enzymatic antioxidants Vitamin E, A and C activities in patients with chronic renal failure before and after hemodialysis.

MATERIALS AND METHODS:

The evaluation was comprised of two groups and total subjects 60. One was control group (males 20, females 10) 30 subjects. The study was carried out after the approval from the MAPIMS&R animal ethical committee(Regd. No. MAPIMS /1068/PO/ac/10/CPCSEA). The control subjects were non-diabetic, non-smoker, non alcoholic and without any chronic diseases and illness. Another group was CRF patients (males 20, females 10) 30 subjects with regular hemodialysis for weekly and attending the dialysis unit for past one year. The CRF subjects urea values were 150 $\pm$ 35 mg/dL and creatinine values were 8.5 $\pm$ 2.5. The CRF patients were not taking antioxidants. Blood samples were collected by venipuncture from the control group and the CRF patients before starting hemodialysis and samples were collected one month later from the same patients.

Determination of Malondialdehyde (MDA) [11]: This method depends on the development of a pink coloured complex between malondialdehyde and thiobarbituric acid (TBA), having a maximum absorption at 532 nm.

Determination of L-carnitine [12]: L-carnitine levels were measured with enzymatic UV-method (Genesis, 340 nm). The reference range for L-carnitine in humans was 3.85 $\pm$ 0.82 mmol/L [16].

Determination of α-Tocopherol [13]: This method is based on the Emmerie Engel reaction. The Xylene extract of plasma containing α-tocopherol reacts with ferric chloride, thus reducing the ferric ions to ferrous ions. The ferrous ions then react with 2, 2' – dipyridyl to give a red coloured complex which is measured at 520 nm. Carotenoids, which were also extracted into xylene, were estimated by their absorbance at 460 nm and a correction was applied at 520 nm. The carotenoid absorption at 520 nm was 29% of the absorption at 460 nm.

Determination of Retinol [14]: Proteins get precipitated on the addition of ethanol and the concentration of Retinol can be determined by reading the extinction value of the heptane extract of retinol at 327nm.

Determination of Ascorbic Acid [15]: When ascorbic acid reacts with 2, 6-dichlorophenol indophenol, reduced 2, 6-dichlorophenol indophenol is formed which is colourless. The decrease in colour is proportional to the concentration of ascorbic acid which is present in the solution. Decrease in the optical density was measured at 520nm and the concentration was calculated from standards which were treated similarly.

Enzymatic Antioxidants Assay:

SOD activity was measured based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol [16]. Erythrocyte Glutathione peroxidase (GSx) determination was performed using erythrocytelysate method [17]. Catalase activity was assayed by the decomposition of hydrogen peroxide by Sinha [18] and reduced glutathione was determined by Ellman method [19].

Statistical analysis: Data are expressed as mean ± SD and analyzed with SPSS program 10. Student's t-test was used to compare the groups. The level of statistical significance was set at $p<0.001$.

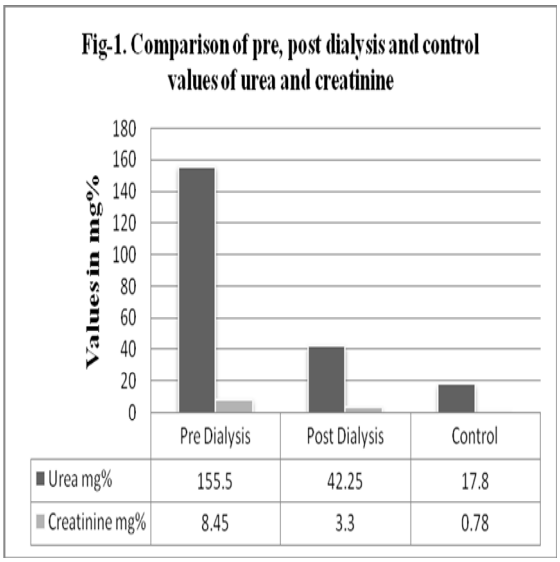
RESULTS AND DISCUSSION:

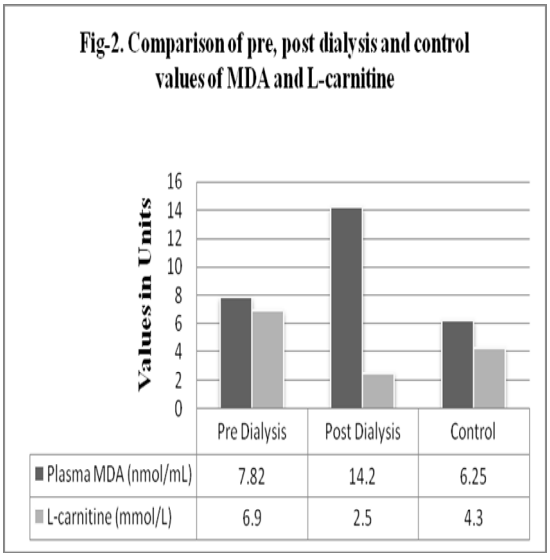
The antioxidant study of CRF patients pre dialysis and post dialysis and control showed in Table 1. The average and standard deviation of plasma urea and creatinine (Fig-1) after the dialysis process were 42.25 ± 7.51mg/dl and 2.05 ± 0.55mg/dl respectively which showed significant reduction in their concentration after Hemodialysis [$p<0.001$]. The levels of MDA were significantly increased in pre-dialysis CRF patients as compared to the controls ($p<0.001$) and the levels were further significantly increased in post-dialysis (Fig-2) patients as compared to the pre-dialysis patients ($p<0.001$). CRF leads to imbalanced production of oxygen free

radicals and cellular antioxidants system. The NADPH required for HMP pathway, which is declined in CRF and leads to extension of free radicals. This increasing the process of oxidative stress and responsible for the augmentation of the disease, thus leading to an increase in MDA levels [20, 21-22]. Further increase in the levels of MDA in post-dialysis CRF patients may be due to the exacerbation of the lipid peroxidation process by hemodialysis. Hemodialysis leads to the activation of complement system, which in turn stimulates free radical generation, mainly superoxides through the NADPH system. Heparin which is used in hemodialysis causes lipolysis, thus increasing free fatty acid in plasma, which leads to the exacerbation of lipid peroxidation. The red cell MDA increases the rigidity of the RBCs and makes them more susceptible to lysis during haemodialysis [23, 24].

Table: 1 Results of CRF patients before and after hemodialysis and control

Parameter	Pre Dialysis	Post Dialysis	Control
Urea (mg/dL)	155.5 ± 7.82	42.25 ± 7.51	17.8 ± 2.02
Creatinine (mg/dL)	8.45 ± 1.85	3.3 ± 2.55	0.78 ± 0.85
Plasma MDA (nmol/mL)	7.82 ± 0.42	14.2 ± 5.05	6.25 ± 0.25
L-carnitine (mmol/L)	6.90 ± 2.5	2.52 ± 1.8	4.30 ± 1.20
SOD (u/ gHb)	982 ± 35	965 ± 42	1195 ± 92
Catalase (u/ gHb)	1.9 ± 1.02	1.2 ± 0.95	7.4 ± 2.55
GTP (u/ gHb)	32.4 ± 9.54	24.7 ± 10.58	40.3 ± 13.25
Reduced GTP (u/ gHb)	15.2 ± 3.92	13.1 ± 4.25	21.5 ± 2 .15
Vitamin E (mg %)	0.61 ± 0.20	0.35 ± 0.1	0.9 ± 0.05
Vitamin A (µg %)	25.5 ± 1.50	16.2 ± 2.10	37.5 ± 2.50
Vitamin C (mg %)	0.63 ± 0.05	0.31 ± 0.07	0.93 ± 0.15
p- Value	< 0.001	< 0.001	-





Existing evidence showed that despite advances in dialysis therapy, a high percentage of patients on maintenance dialysis therapy, suffered from complication of hemodialysis [25]. The study of Evans et al showed, that the average of predialysis serum L-carnitine concentration was 19.5 µmol/l, where the post dialysis level was 5.6 µmol/l [8]. Also, Alhomida's research indicated that the average of serum L-carnitine before and after hemodialysis was 42 ± 6.3 µmol/l and 17.1 ± 6.3 µmol/l, respectively [26]. The results of this study were consistent with the studies of others and showed a decrease of L-carnitine after hemodialysis (Fig-2). L-carnitine is a small water-soluble molecule, therefore it is freely dialyzed because of a molecular weight gradient; the acyl-carnitine moieties are less likely to be filtered by the membrane than free carnitine [8, 27]. Accumulation of acyl-carnitine is belived to contribute directly to arrythmogenesis [28]. This accumulation also alters mitochondrial membrane permeability and has been suggested to promote apoptosis. Altered membrane permeability has been implicated to modify the activity of various hormone receptors, including insulin receptors [29].

Fig-3. Comparison of pre, post dialysis and control values of SOD

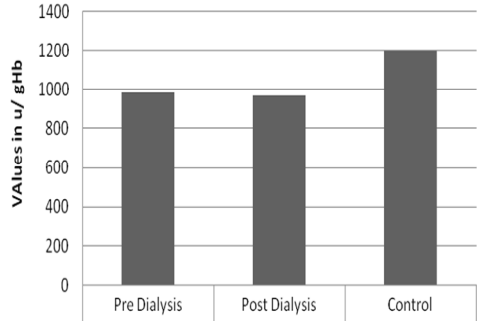
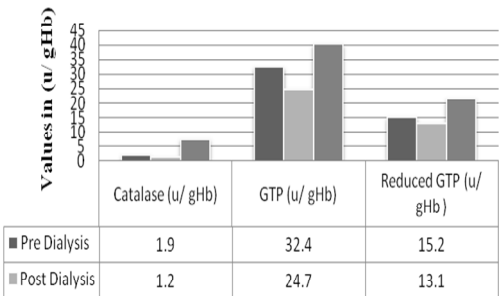


Fig-4. Comparison of pre, post dialysis and control values of Catalase, GTP and reduced GTP

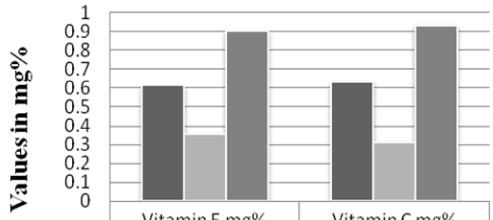


Antioxidant enzymes such as superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase were significantly decreased in pre dialysis patients (Fig-3, 4) as compared to the controls (P<0.001) and the values further significantly decreased in post dialysis patients as compared to the pre dialysis CRF patients (P<0.001). Weinstein et al [30] reported an increased erythrocyte activity of glutathione peroxidase. In our study we have obtained decreased erythrocyte catalase activity in post dialysis when compared with pre dialysis and control group. Hence the results shows that significant difference of antioxidant enzymes among pre and post dialysis group related with the loss of antioxidant enzymes through the membrane and the decreased antioxidant enzymes cause to increase of lipid peroxidation.

The plasma concentrations of Vitamin E, Vitamin A and Vitamin C were significantly decreased. The levels were further significantly decreased in postdialysis CRF patients (**Fig. 5, 6**) as compared to predialysis CRF patients (P<0.001). The low levels of α-tocopherol leads to increased lipid peroxidation and also interfere the lipid metabolism. The absorption of α-tocopherol reduced due to altered lipid metabolism. After dialysis, further decreased levels may be due to increased consumption in an attempt to reduce the effect of the oxygen free radicals [31-33]. Decreased levels of Vitamin A in predialysis CRF patients as compared to healthy controls, was due to enhanced lipid peroxidation, thus leading to the exhaustion of hepatic storage. So, there was reduced conversion of β-carotene to vitamin A and decreased secretion of vitamin A from the liver into the circulation.

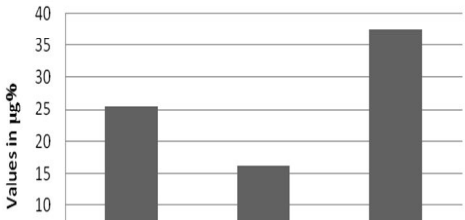
In post-dialysis CRF patients, the enhanced production of oxygen free radical leads to the increased utilization of vitamin A and its microsomal degradation, thus resulting in its reduced levels [23]. Low levels of Ascorbic acid in predialysis CRF patients as compared to controls, may be due to the utilization of ascorbic acid to generate α- tocopherol from the α-tocopheroxyl radical at the water lipid interface. The ascorbate may be reduced because it is an efficient quencher of superoxide peroxyl and hydroxyl radicals. In addition to the above, there may be related nutritional deficiency of ascorbate to limit potassium intake in CRF patients. During haemodialysis, there may be additional loss of ascorbic acid from plasma as it is a water soluble vitamin [32].

Fig-5. Comparison of pre, post dialysis and control values of Vitamin E and C



	Vitamin E mg%	Vitamin C mg%
Pre Dialysis	0.61	0.63
Post Dialysis	0.35	0.31
Control	0.9	0.93

Fig-6. Comparison of pre, post dialysis and control values of Vitamin A



	Pre Dialysis	Post Dialysis	Control
Vitamin A µg%	25.5	16.2	37.5

CONCLUSION

In conclusion, the result of the present study apparently indicates that in CRF patients undergoing hemodialysis, oxidants and antioxidants play a vital role in the pathogenesis of disease. In this study CRF patients suffered from carnitine deficiency before hemodialysis and serum L-carnitine decreased markedly after hemodialysis ($P<0.001$). In these patients, oxidative stress is further exacerbated by hemodialysis, as evidenced by increased lipid peroxidation ($P<0.001$). Therefore oxidative stress management and supplementation with L-carnitine, parallel to other therapeutic agents, may improve condition of hemodialysis patients. The decreased levels of antioxidants like Vitamin E, Vitamin A and Vitamin C shows the increase in oxidative stress. Inflammatory processes during dialysis appear to be the main factors which are involved in oxidative stress. Exogenous supplementation of antioxidants may decrease the damage to renal tissue by quenching and preventing the free radical action which are responsible for the disease process. The new methodology or sophisticated membrane to be invented for hemodialysis and further it will develop for removing ROS without disturbing any antioxidants, it will help improvement life of CRF patients.

ACKNOWLEDGEMENT

The authors wish to express their acknowledgement to the Management MAPIMS&R, Prof & H.O.D, Dept. of Nephrology, For their constant help throughout the study.

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