Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i4.6039

ASSESSMENT OF PROGNOSTIC FACTORS ASSOCIATED WITH ANTI-OXIDATIVE STATUS IN END STAGE RENAL DISEASE PATIENTS EXPERIENCE SURGICAL TRIAL

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ABSTRACT

Background: End Stage Renal Disease (ESRD) or Chronic kidney disease (CKD) is a group of diverse deformities that upshot the physiology and anatomy of the kidney. Kidney failure is the most severe outcome of the chronic kidney disease.

Methodology: Comparative study. Sixty patients of End Stage Renal Disease (ESRD) and Fourty five age and gender matched clinically evidently fit persons were entitled for incorporation in the study. 5 ml blood sample were drawn and subjected to centrifuge at 4000-5000 rpm for 15 minutes for the separation of serum. Serum MDA, SOD, CAT, GSH, VIT A, VIT C, VIT E, Nitric oxide (NO), Neuraminidase, Electrolytes TNF-alpha and IL-2 were estimated. **Results:** Serum Sodium (Na^+) level in the ESRD patients was elevated (179.53±3.26) as compared to control (147.26±4.26) and significant statistically (0.04<0.05). Serum Potassium level (K^+) was also raised in the ESRD patients (16.12±5.26) as compared to control group (11.09±3.26) and also statistically substantial (0.03<0.05). Vitamin A level in ESRD patients was decreased (87.99±5.26) as compared to healthy subjects (102.25±14.26) and statistically substantial (0.002<0.05). MDA level shows elevated level in ESRD patients (8.06±1.5) as compared to control (1.25±0.65) and statistically significant (0.045<0.05).

Conclusion: Present study showed that there is a correlation exist between Oxidative stress, Vitamins, Electrolytes, IL-2, TNF-alpha and CKD. These results indicate a perfect description related to circulating biomarkers and lipid peroxidation. Increased level of MDA as a biomarker of lipid peroxidation, increased IL-2 and TNF-alpha and Nitric oxide (NO) level is the cause for the progression of the disease.

Key words: *CKD, MDA, SOD, CAT, IL-2, TNF-ALPHA*

INTRODUCTION

End Stage Renal Disease (ESRD) or Chronic kidney disease (CKD) is a group of diverse deformities that upshot the physiology and anatomy of the kidney. The disease expression changes are associated with cause and pathology, severity of disease and progression rate of the disease. Conceptual model, definition, staging of chronic kidney disease were introduced 10 years before (1-4). it is recognized as a life threatening disorder recommended by guidelines to the affected people who need care by nephrologists (5). No doubt these strategies have an important effect in different way but also produced controversy (6).

Most common and severe outcome of chronic kidney disease (CKD) is the kidney failure and their symptoms are due to the reduced kidney function. In case of harshness, it can be cured by the dialysis or transplantation and this stage is known as end stage renal disease (ESRD). When GFR level is less than 15mL/min per 1.73m2 it refers to kidney failure and at this stage transplantation or dialysis is required. Decreased GFR complications i.e. high risk of cardiovascular disease, acute kidney injury (AKI), infections, cognitive deterioration and physical functions also refers to outcomes of CKD (7-11). CKD is related to age, diabetes, hypertension, obesity and cardiovascular disease in the developed countries and also associated with glomerulosclerosis and hypertensive nephrosclerosis (12).

About 8 million adults are affected from chronic kidney disease in United States of at least stage 3 (glomerular filtration rate (GFR) of below 60 ml/min/1.73m^{2}of body surface area) (13) is reported about the rates of death, cardiovascular disease. It was noticed that in the previous studies that elevation in the serum creatinine level from mild to moderate are the cause of increased rate of death (14-16) and also from the cardiovascular cause (17) but it also notify that chronic kidney disease itself increases the cardiovascular disease risk that has not been recognized (18,19).

Inadequacy of health care resource programs about kidney replacement therapy is the huge distinction in the pervasiveness or popularity of ESRD amongst more and less developed countries. ESRD patients are under the burden of CKD due to the earlier stages of disease exceed by 50 times those ESRD (13). Systemic hypertension is the most notable factor among the modifiable progression factor (20). Most reliable and powerful predictor for the severity of CKD is proteinuria (21). It also conquer that proteinuria is causative factor in the progression of clinical nephropathies. Diseased persons that have elevated urinary protein excretion rate (>3-5 g in 24 h) have high progression rate as compared to lower to moderate proteinuria patients (<1-3 g in 24 h) (22).

Depleting lymphocytes, blocking lymphocyte response pathways can be used to achieve immunosuppression. Therapeutic effect (suppressing rejection), undesired consequences (infection or cancer) non-immune toxicity are the different effects of the immunosuppressant. Infection and cancer are caused by the immunodeficiency like post-transplantation lympho-proliferative disease (23) that associated to the intensity of immunosuppression. Cyclosporine is dissolved in an olive oil based solution for oral use while for the intravenously, alcohol solution and castor oil used. Both methods have equal effect while intramuscular administration is avoided in case of humans (24). Cyclosporine may be administered by intravenously if the patient unable to tolerate through orally. There are many sides effects related with the use of cyclosporine, nephrotoxicity is one of them and may cause due to the long-term utilization of immunosuppression. Renal dysfunction also induced by the cyclosporine. Many other nephrotoxic effects are hyperkalemia and hypertension. Cyclosporine therapy also cause Hirsutism about 30% to 44% of the patients (25).

Disruption in the systematic cellular and molecular role that occurred by disproportion among reactive species and natural antioxidant capacity of cell is refer to oxidative stress. Oxidative stress is progressed due to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and has free radicals in the biological system. Superoxide (O_2-), hydroxyl radical (OH-) and hydrogen peroxide (H_2O_2) are the reactive oxygen species. Cellular localization and availability of antioxidant enzymes and thiol are in balance with the production of ROS. Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) are the anti-oxidative enzymes. GSH is synthesize in the presence of ATP while its lowering capacity depend upon NADPH and pentose

phosphate pathway. Advanced oxidation protein products (AOPP) deposited in serum of the CKD patients suffered from uraemia and diabetes (26).

Objective

To investigate the role of anti-oxidative biomarkers, Micronutrients, serum cytokines in end stage renal disease (ESRD) patients.

METHODOLOGY

Whole experimental work was done after the approval of research and Ethical committee, Minhaj University Lahore.

Source of data

I. Sixty patients of End Stage Renal Disease (ESRD) were suitable for inclusion in the study at Jinnah Hospital Lahore. Elaborated history, clinical impediments if any behaviour in certain smoking and tobacco mastication were assembled from subjects of the study, by presenting a questionnaire.

II. Fourty five age and gender matched clinically evidently fit persons were entitled for incorporation in the study as controls.

Data Collection

Blood specimens were assembled with sterile precaution. Informed consensus from subjects was acquire earlier collection of blood specimen.

Specimen processing

Specimen of Patients and controls was collected and processed. 5ml blood specimen was drawn in EDTA-Vial and centrifuged at 4000 rpm for 15 minutes.

Reagents

All reagents are of analytical grades, purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Following Parameters Were Estimated

Reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), electrolytes sodium (Na^+) and potassium (K^+) concentration, Nitric oxide (NO), neuraminidase, interleukin-2 and tumor necrosis factor (TNF-alpha). Estimation of vitamins (Vit A, C and E). MDA was estimated spectrophotometricaly through the method of Ohkawa *et al* (27). SOD was estimated through the method of Kakkar *et al* (28). Activity of CAT was observed through the method of Aebi, (29). GSH by Moron *et al.,* (30).

RESULTS

Table 1: Estimation of Oxidative stress Biomarkers in ESRD patients and Normal Subjects

VARIABLES	CONTROL (n=45) (Mean ±S.D)	SUBJECTS (n=60) (Mean ±S.D)	P<0.05
MDA (nmol/ml)	1.25±0.65	8.06±1.5	.0458
GSH (µg/dL)	8.64±.025	3.25±1.05	.03.25
SOD (µg/dL)	0.99±.06	.065±.0015	.0114
CAT (µg/dL)	3.19±.054	1.22±.012	.035
AOPPs	1.09±0.02	3.25±0.065	.0324
NITRIC OXIDE (NO)	13.26±1.25	42.15±5.26	.0225

Data given in table 1 shows the fine depiction of different biomarkers of oxidative stress evaluated in ESRD patients. When biomarkers of oxidative stress were evaluated, elevation in MDA level was detected in ESRD patients (8.06) as related to control ones (1.25) and statistically substantial (0.045<0.05). Serum GSH level in ESRD patients decreased highly (3.25) as compared to healthy ones (8.64). Serum SOD level in ESRD patients shows decline (0.065) as compared to control group (0.99) showing that the data is statistically substantial (0.01<0.05). Serum Catalase (CAT) level in ESRD patients was observed (1.22) in contrast to control group (3.19) and statistically momentous (0.03<0.05). When AOPP level was checked in ESRD patients, the observed value was greater (3.25) from healthy ones (1.09) and statistically momentous (0.03<0.05). Nitric oxide level in the ESRD patients was increases (42.15) as compared to control (13.26) and statistically significant (0.02<0.05).

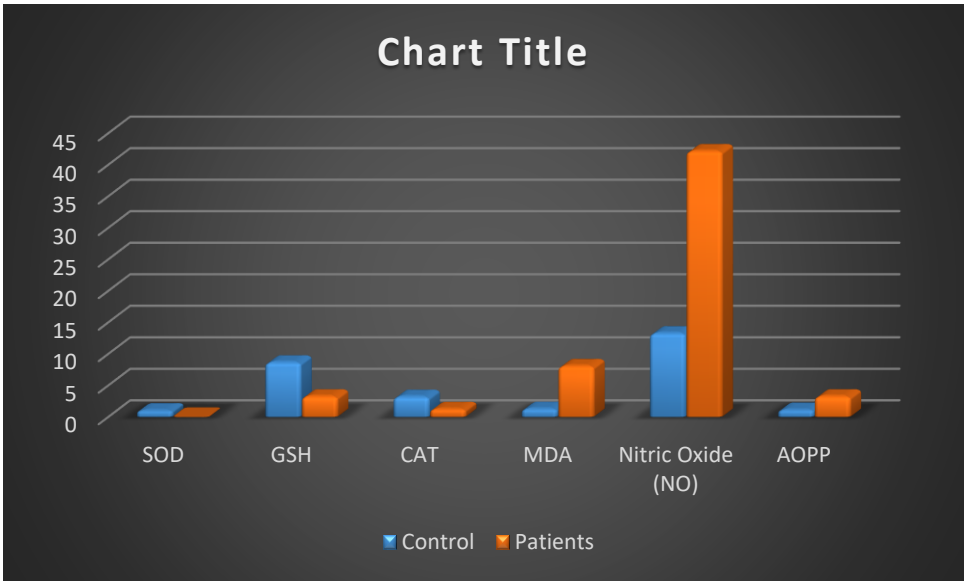

Fig 1: Graphical representation of Oxidative Stress biomarkers in ESRD and Normal Subjects

VARIABLES	CONTROL (n=45) (Mean ±S.D)	SUBJECTS (n=60) (Mean ±S.D)	P<0.05
Na^+ (mg/L)	147.26±4.26	179.53±3.26	.045
K^+ (mg/l)	11.09±3.26	16.12±5.26	.033
Vit.A(µg/ml)	102.25±14.26	87.99±5.26	.0024
Vit. E (µg/ml)	6.35±1.22	2.15±0.25	.026
Vit.C(µg/ml)	3.29±0.25	0.965±.095	.0214
IL-02 pg/ml	219.65±15.26	402.5±18.26	.0024
TNF-α (pg/ml)	18.65±2.25	37.26±4.26	.0016
NEURAMINIDASE	7.26±2.16	19.26±3.25	.0014

Table 2: Estimation of micronutrients, serum electrolytes and IL level in Normal and disease persons

The data described in the above table shows the fine depiction of different parameters evaluated in the patients distress from chronic kidney disease (CKD) or End Stage Renal Disease (ESRD).
When the electrolyte balance (Na^+ and K^+) was estimated, serum sodium level in ESRD patients increases remarkably (179.53) as compared to healthy ones (147.26). Serum potassium level in CKD patients was (16.12) while in control group was (11.09). This shows that the data is statistically significant (0.045<0.05 and 0.033<0.05 respectively).
Similarly when the level of vitamins was measured, vitamin A level in ESRD patients decreases (87.99) as compared to control ones (102.25) and statistically significant (0.05>0.002). Serum

vitamin E level in ESRD patients was (2.15) while in healthy individuals was (6.35). This represents that the data is statistically significant (0.02<0.05). Serum Vitamin C level in ESRD patients was measured as (0.965) while in control group was (3.29) while means that the value decreases remarkably.

Interleukin-2 (IL-2) level in ESRD patients was estimated as (402.25) while in healthy individuals as (219.65) showing statistically significant data (0.00<0.05). TNF-α level in ESRD was (37.26) while in control ones (18.65).

Neuraminidase level in ESRD Patients was (19.26) while in control it was (7.26) and it was statistically significant (0.001<0.05).

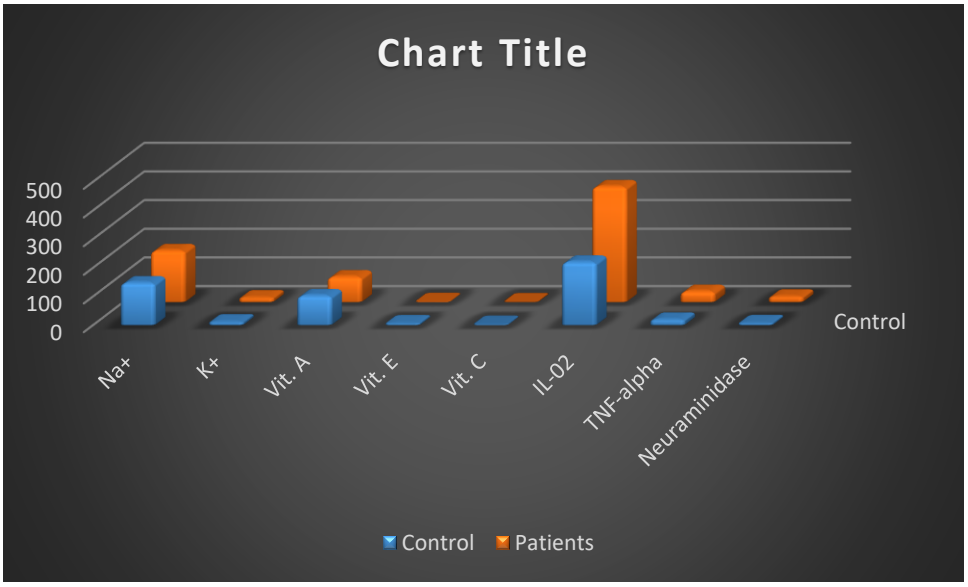

Fig 2: Comparison of micronutrients, serum electrolytes and IL level between Normal and disease persons

DISCUSSION

Renal transplantation is used as a treatment of end stage renal disease (ESRD) patients. First renal transplantation carried out since 1955 and has been continues struggle to improve the long and short term survival of renal transplantation. Cyclosporine was introduced in 1980 to prevent the acute rejection rate. The presence of pre-existing lesions in the donor is one of the important factor in the evaluation of chronic pathologic damage and immunosuppressive therapy effect. These lesions correlated to the donor's age and existence of many other pathologic conditions like diabetes mellitus and arterial hypertension. Hemodialysis can be consider additional stimulus for the ROS production in hemodialysis patients. It happened due to the activation of inflammatory cell that are caused by biocompatible membrane insufficiency that improve by many bacterial products passed across from dialysate to the blood compartment and releasing of ROS by neutrophils may stimulate by it. Oxidation of LDL and oxidized LDL can also be stimulate from ROS that is not predictable by LDL receptors. Endothelial dysfunction and left ventricular hypertrophy is caused by the oxidative stress. Although quantification of oxidative stress remain difficult task due to lack of standardization tests, increased oxidative stress is noticed in the ESRD patients.

Chronic rejection considered major reason of graft loss in long-term studies. Pervasiveness of chronic rejection varies between 15 to 85% based on follow up duration. Acute rejection reflected as major interpreter of chronic rejection. It was noticed that white recipients have less chronic rejection as compared to black transplant recipients. Graft survival rate has improved by the high dose of cyclosporine, high bioavailability of cyclosporine related to the reduced episodes of acute rejection.

Present study shows the decline in the anti-oxidants and elevation of the cytokines and nitric oxide level in the renal transplant patients treated with the cyclosporine. Data obtained from different

studies shows that immunologic factor like prolonged cold ischemia, non-immunologic factors. Reactive oxygen species (ROS) oxidize the non-enzymatically arachidonic acid in to lipoproteins that cause production of vasoconstrictive pro-inflammatory products like isoprostane. Different studies suggested that inverse relationship exist between oxidative stress biomarkers and GFR due to which ROS increase in gradually as renal function depreciate.

Conclusion

Multiple conditions i.e. diabetes, dyslipidemia, and hypertension are strongly associated with the progression of oxidative stress. Present study concluded that Strong association exist between Oxidative stress, Immunosuppressant, Micronutrients and electrolyte balance in ESRD patients. Increased Lipid peroxidation leads to elevated level of MDA remarkably whereas Anti-oxidants decreases. Elevation in the Nitric oxide, cytokines (IL-2, TNF-alpha) and decrease level of vitamins are the cause for the progression of End Stage Renal Disease (ESRD) or CKD or Renal Failure.

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