ANTIOXIDANT LEVEL IN NORMAL AND DIALYZED PATIENTS USING FRAP METHOD

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ABSTRACT

To examine the serum antioxidant levels like vit-C, vit-E and glutathione in patients with renal diseases who were subjected to dialysis and to evaluate the antioxidant by FRAP method. To find out the involvement of free radicals in pathogenesis of renal disease. Fifty patients with high levels of creatinine and urea level were included in the study of dialysis. A difference of antioxidant level of vit-C, vit-E and glutathione was observed. The study therefore suggests the importance of assessing these marker oxidative stress antioxidant capacities in renal disease during dialysis.

Keywords: FRAP (Ferric Reducing Ability of Plasma), TAC (Total Antioxidant Capacity), GSH (Glutathione).

INTRODUCTION

Total antioxidant capacity is a parameter characteristics activities of antioxidant status of the body and reflect the oxidative stress imposed on the organism (Caos and Prior, 1999; Bartosz, 2003). There is growing interest in the role of free radical damage in the neopalsia, vascular disease, neurodegenrative disease and aging (Hallwell, 1994). Oxidative stress occurs in a cellular system when the production of free radical moieties exceeds the antioxidant capacity of that system. If cellular antioxidant do not removed free radicals attack and damage protein lipid and nucleic acids. The oxidized nitrosylated products of free radical attack have decreased biological activity, leading to loss of energy metabolism, cell signaling, transport and other major function, these altered products are also targeted protesome degradation, further decreasing cellular function. Accumulation of such injury ultimately lead a cell to die through necrotic or apoptoic mechanism.

Glumerular dysfunction may be due to disturbances in lipoprotein metabolism. A positive association between urinary albumin excretion and serum total cholesterol (Jensen *et al*., 1988) and apolipoprotien serum apolipoprotien B level, have been reported to be predictive of the development of microalbuminurea, but an increased in plasma cholesterol will be onset of proteinurea has also been described (Jensen *et al.,*1987).

Nephropathy is characterized by persistent albuminurea, a decline in the glumerular filtration rate and evaluated arterial blood pressure (Cross *et al.,* 2005) and is the leading cause of chronic renal failure (Nakai *et al.,* 2005). Oxidative stress was reported to play a key role in pathogenesis of many diseases including nephropathy (Jee *at al*., 2005).

Patient on maintenance dialysis are at risk of oxidative stress due to an imbalance between pro and antioxidant factors (Cristol and Canauld, 1994). By stimulating free radical production or elimination of anti oxidants, dialysis may contribute to deducing antioxidative defence. Our study was design to compare oxidative stress before and after single session to compare oxidative stress before and after single dialysis session.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were analytical grade purchased from Sigma, Aldrich and Fluka. Urea, Creatinine kit provided by Randox Lab., U.K.

Patients

50 patients with chronic renal failure were included in studies, dialysis was performed using a dialyzer made of regenerated cellulose did not have any infectious disease. The patients did not show sign of iron overload and did not use vitamin supplements or other agents with antioxidants effects. The study was approved by the local ethics committee of Gomal Teaching Hospital.

A total of 50 age and sex- matched volunteers were also examined. The mean age was 65 years (range 40-83years).50 control subjects were in static condition and did not take vitamin supplements or any other agent with antioxidants effects.

Laboratory investigations

The blood samples were withdrawn before and after dialysis from an arteriovenous fistula into test tubes. Blood samples were processed in a laboratory

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Table 1: Serum biochemical parameters before and after dialysis

Parameters	Normal	Before dialysis	after dialysis
Urea (mM/L)	117.76 ± 13.97	520.26 ± 93.86	225.21 ± 25.9
Creatinin (µM/L)	62.82 ± 9.04	878.47 ± 224.28	180.42 ± 35.13
Renal Clearance ML/Minute	123.38 ± 14.95	7.34 ± 2.79	75.35 ± 10.23
FRAP Vit C µM/L	436.44 ±44.65	336.35 ± 46.79	336.35 ± 16.79
FRAP Vit E µM/L	481.36 ± 49.24	371.41 ±5 1.91	395.314 ± 41.34
FRAP GSH µM/L	2407.02 ± 246.25	1275.92 ± 259.52	1857.33 ± 259.5

Data are expressed as mean ±SEM of 50 normal and 50 renal patients. Student t-test $p<0.05$ significantly different from normal control group.

immediately after collection. Total antioxidant capacity was estimated by modified FRAP assay which depends upon the reduction of ferric tripyridyltriazin [Fe (III)-TPTZ] complex to ferrous tri pyridyl triazin [Fe (II)-TPTZ] at low pH. [Fe (II)-TPTZ] has an intensive blue color can be monitored at 593 nm. The assay response was standardized against the antioxidant standard Trolox.

Statistical analysis of data

The values are expressed as mean ± SEM, the statistical analysis of data was performed using the T-test value $p<0.05$ were considering significantly.

RESULTS

Table showed that serum urea and creatinine levels were significantly elevated in renal disease as urea levels elevated highly significantly as compared with normal control as 520± 93.8 mM/l before dialysis, after dialysis this value was found to be the 225.21 ± 25.8 mM/l. The serum creatinine was also highly significant increased as compared with normal control before dialysis which was 878.4 mM/l and decline after dialysis 180.4 mM/l. Blood urea and creatinine levels declined during dialysis renal clearance reached near normal level.

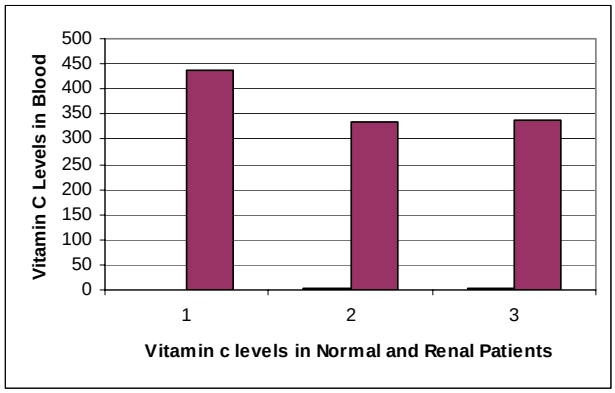

Fig. 1: Shows comparison of vitamin c level in normal and renal patients.
On X-axis 1= Normal Subjects, 2= Before Dialysis 3= after dialysis

The antioxidant vitamin C and vit E were determined by FRAP modified methods the values are shown in table. Fig. 1 shows the comparison of vit c level in normal control and renal patients. The mean± SD serum vit C level decreased considerably before dialysis 250.5 mM/l as compared normal control ($p<0.05$ by students T-test). However the mean values of antioxidants in patients after dialysis was calculated as 336.4 mM/l which is also less then normal value which can reach to normal level with vit C therapy.

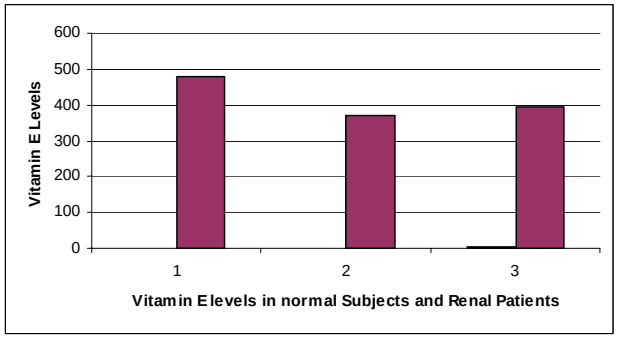

Fig. 2: Shows comparison of vitamin E level in normal and renal patients.
On X-axis 1= Normal Subjects, 2= Before Dialysis 3= after dialysis

The FRAP vit E mean ± SD serum level was 481.3 ±49, 371.4±51 and 395.3±4 for normal control renal patients before and after dialysis as shown in fig. 2. The mean ± SD vit E level was considerably less then normal level. No significant decrease was observed in dialysis. Fig. 3 showing comparison of glutathione (GSH) level in normal and renal disease patients before and after dialysis. The mean ±SD of GSH levels in control are found to be 2407±246 mM/l and in renal patients before e dialysis were 1275.1±135 mM/l and after dialysis 1857±259 mM/l. These level were increased after removal of toxic compound of Glutathione which is one of the most important defense system have free radical scavenging effects.

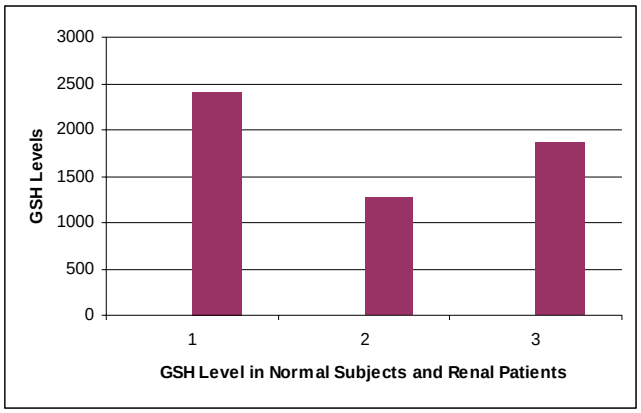

Fig. 3: Shows comparison of Gsh level in normal and renal patients.
On X-axis 1= Normal Subjects, 2= Before Dialysis 3= after dialysis

DISCUSSION

Increased serum oxygen free radicals can induce glumerulosclerosis and chronic tubulointestitial damage in the kidney. A progressive decline in the glumerular filtration rate due to loss of functioning nephrons and histological renal damage are common characteristics in the development of nephropathy.

As a result of free radical production the vit-E is converted into α-tocopheroxyl free redical. The α-tocopheroxyl free radical dose not possess an antioxidant properties and even tigger the process of lipid per oxidation. Vit C is a substance responsible for the regeneration of the α-tocopheroxyl free radical back to tocchopherol a continuous supply of vit C into the arterial blood line during dialysis.

As demonstrated by most studies patient on dialysis do not show vit E deficiency and vit E levels don't decline during dialysis (Hegbrant, 1999). Vit C deficiency is frequent in the dialysis population by contrast and dialysis associated with significant losses of vit C (Bohm *et al*., 1997). Single vit C infusion resulting in maintaining its levels at values comparable to pre dialysis levels. The potent antioxidant activity of vit C maintains cellular GSH conserve the integrity of biomembrans and reduce leakage of cytosolic lactic dehydrogenase in liver. Moreover, it may diminish primary DNA damage of cells (Benkovic *et al.,* 2008).

In conclusion the antioxidant effect of vit E and vit C can decrease metabolic disturbances and oxidative stress that are associated with nephropathy. Consumption of effective antioxidant therapy may delay the onset and progression of nephropathy and renal function impairment.

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