

Relation of oxidative stress in chronic renal Failure patients – A study

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Abstract

Background: Chronic kidney disease is a devastating disease with clinical and ethical dimensions and major public health problem globally. The cause for CRF include obstructive uropathy, primary glomerular diseases, reflux nephropathy, hypoplastic or dysplastic kidneys. Antioxidants control the oxidative stress caused by reactive oxygen species, and act as major line of defence, regulating overall health.

Aim:

1. To evaluate oxidative and antioxidant status in chronic renal failure disease.
2. To evaluate its inflammatory response in oxidative stress.

Methods:

1. Samples were collected from Wenlock District Government Hospital, Mangalore (25 patients with chronic renal failure, 25 normal patients) from Aug. 2007 to Aug. 2008.
2. The Parameters estimated are in plasma (total thiols, total proteins, antioxidant activity, advanced oxidation protein product). In red blood cells suspension % hemolysis, in whole blood (Glutathione), in serum, serum iron.
3. Statistical analysis was done by Mann Whitney U test to determine the significant of difference between two means.
4. Informed and written consent was taken from patients and ethical committee clearance was obtained.

Results:

I. Non enzymatic antioxidants and total antioxidant activity (TOA):

Whole blood glutathione (GSH), Plasma total thiols, Albumin decreased

II. Markers of oxidative damage:

Advanced oxidation protein products, Iron increased, Percentage hemolysis no increase

III. Plasma proteins as inflammatory markers:

Total proteins decrease, Globulin increased

Conclusion:

- In our study the non-enzymatic antioxidant metabolites were found to be decreased in chronic renal failure disease.
- There was very highly significant decrease blood glutathione, plasma total thiols and albumin in chronic renal failure disease.

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- No significant increase in advanced oxidation protein products (AOPP), % hemolysis.
- Very high significant increase in serum iron in patients of chronic renal failure disease.
- There is oxidative stress in patients of chronic renal failure disease.

Key words: Oxidative stress, chronic renal failure, oxidative damage.

Introduction

Chronic kidney disease (CRD) is a devastating disease with clinical, economic and ethical dimensions and is emerging as a major public health problem globally^[1]. Chronic renal failure (CRF) is the irreversible deterioration of renal function that gradually progresses to end stage renal disease (ESRD)^[2]. Incidence of end stage renal disease (ESRD) is showing an alarming 8 percent annual growth rate. With an estimated end stage renal disease (ESRD) of 100 per million people there could well be about 100,000 patients per year in India^[1].

The chief causes of chronic renal failure include obstructive uropathy, primary glomerular diseases, reflux nephropathy and hypoplastic or dysplastic kidneys. Progressive hyperperfusion and hyperfiltration causes increasing glomerular injury and further renal damage. Symptoms of chronic renal failure are usually seen when glomerular filtration rate (GFR) is between (GFR < 15 mL/min/1.73 m²) 10-25% of normal. Once end stage renal disease (ESRD) supervenes, renal replacement therapy in the form of chronic peritoneal or haemodialysis and transplantation is necessary^[2].

Reactive oxygen species could be important causative agents of a number of human diseases^[3]. Thus, antioxidants which control the oxidative stress state represent a major line of defense, regulating overall health^[3]. Human plasma contains many different non-enzymatic antioxidants. The antioxidant status in human plasma is dynamic and may be affected by many factors. Thus, the relationship between non-enzymatic antioxidant capacity of plasma and their levels which are well known markers of oxidative stress (activity of plasma oxidized protein, lipid hydroperoxides, total thiol groups, glutathione level and plasma iron level) reflect health status^[4-6].

Disturbances in the antioxidant system could play a role in pathogenesis

Inadequate removal of reactive oxygen species (ROS) may cause cell damage by attacking membrane

lipids, proteins and inactivating enzymes thus mediating several forms of tissue damage. The aim of our study was to evaluate the levels/activities of non-enzymatic antioxidants in blood of patients.

Aim

1. To evaluate oxidative and antioxidant status in chronic renal failure.
2. To evaluate its inflammatory response in oxidative stress.

Materials and methods

A. Criteria for selection of subjects for study

Test samples were collected from 25 patients aged 20 to 60 years, both male and female having chronic renal failure on clinical basis.

Control group samples were collected from age and sex matched 25 healthy individuals who are devoid of any diseased conditions like hypertension, diabetes mellitus, epilepsy, psychiatric disorders or history of any drug intake, alcoholism. Samples were collected from Wenlock District Government Hospital, Mangalore from Aug. 2007 to Aug. 2008.

B. Sample Collection and Processing

6 ml blood was collected under aseptic conditions from both patients and normal persons after obtaining the informed consent. 3 ml of blood was collected without anticoagulant to separate serum. The remaining 3 ml of blood was collected with anticoagulant (EDTA). 0.2 ml of whole blood was taken for glutathione estimation and the remaining volume was used to separate plasma. The separated red blood cells (RBC) were washed thrice with cold normal saline (NS), after which they were suspended in an equal volume of normal saline. This 50% RBC suspension was used for estimation of % haemolysis.

C. The following parameters were estimated

1. In Plasma

- a. Total thiols by Ellman method.
- b. Total proteins, albumin, globulin and A : G ratio

by Lowry's method

- c. Total Antioxidant activity by Koracevic method.
- d. Advanced oxidation protein products by modified Witko method.

2. In RBC suspension

hemolysis by Kartha and Krishnamurthy method.

3. In whole blood

Glutathione by Ernest Beutler method.

4) In serum

Serum iron estimation by Kitzes method.

Statistical analysis:

Statistical analysis was done by Mann Whitney U test to determine the significance of difference between the two means.

Results

Non enzymatic antioxidants and total antioxidant activity (TOA):

Whole blood glutathione

Table 1. In comparison to controls, decrease in glutathione (GSH) activity in chronic renal failure is very highly significant.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	45.0532 © 10.4656		
Chronic Renal Failure	25	32.1660 © 15.1402	.004	HS

N = Number of samples, SD = Standard Deviation, P = Probability of chance of significance of difference between two means, S = Significant, HS = Highly significant, VHS = Very highly significant, NS = Not significant.

Plasma total thiols

Table 2. In comparison to controls, decrease in total thiols in plasma of chronic renal failure patients, is very highly significant.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	.5171 © .1180		
Chronic Renal Failure	25	.3238 © .0830	<.001	VHS

Albumin

Table 3. There is a very highly significant decrease in albumin level in chronic renal failure when compared to the controls.

Study group	N	Mean © SD (g/dl)	P	Remarks
Controls	25	4.6440 © .7906		
Chronic Renal Failure	25	3.1875 © .6917	<.001	VHS

Total antioxidant activity

Table 4. The decrease in total antioxidant activity is not significant in patients of chronic renal failure when compared to controls.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	1.0098 © .0593		
Chronic Renal Failure	25	.9750 © .0685	.052	NS

Note: This method of estimating total antioxidant activity (TOA) was not performed in samples of patients having chronic liver disease because high bilirubin levels interfere with the assay.

Markers of oxidative damage

Advanced oxidation protein products (AOPP)

Table 5. The increase in AOPP in patients of chronic renal failure is not significant when compared to controls.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	.1257 © .0539		
Chronic Renal Failure	25	.1291 © .0861	.877	NS

Table 6. Percentage hemolysis, in chronic renal failure shows significant increase at 0 hour, when compared to the control. Whereas, 2 hour sample shows no significant increase when compared to the controls.

Hemolysis at 0 hour in (%)

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	5.2772 © 2.8220		
Chronic Renal Failure	25	9.8828 © 6.5703	.013	S

Hemolysis at 2 hours (%)

Table 7. Percentage hemolysis, in chronic renal failure shows significant increase at 0 hour, when compared to the control. Whereas, 2 hour sample shows no significant increase when compared to the controls.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	10.3671 © 3.0950		
Chronic Renal Failure	25	12.2647 © 5.9393	.169	NS

Iron

Table 8. There is decrease in the serum iron level in the patients of chronic renal failure when compared to controls, the decrease is not significant

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	126.9824 © 34.6687		
Chronic Renal Failure	25	117.5717 © 41.8219	.358	NS

Plasma proteins as inflammatory marker Total proteins

Table 9. The decrease in serum total protein is very highly significant in chronic renal failure, when compared to controls. Decrease in albumin levels and increase in globulin levels is highly significant compared to controls.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	7.8280 © 1.0321		
Chronic Renal Failure	25	6.8750 © .4302	<.001	VHS

Albumin

Albumin has already been discussed.

Globulin

Table 10. Increase in serum globulin is significant in chronic renal failure when compared to controls.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	3.0666 © .6856		
Chronic Renal Failure	25	3.4956 © .4733	.026	S

A : G Ratio

Table 11. Change in A : G ratio is very highly significant in chronic renal failure, when compared to control.

Study group	N	Mean © SD (mmol/L)	P	Remarks
Controls	25	1.5176 © .4783		
Chronic Renal Failure	25	.9210 © .2515	<.001	VHS

Discussion

In a recent study it was calculated that Sulfhydryl groups contribute 52.9% to the measured serum total antioxidant capacity in healthy subjects^[7]. Reduced glutathione levels in uremic patients due to inhibition of glucose-6-phosphate dehydrogenase by uremic toxins has been reported by several investigators.

Plasma thiols have been the objective of growing interest because mild degree of homocysteinemia is associated with an increased risk of developing occlusive vascular disease^[8]. Homocysteine contains a reactive sulfhydryl group and can undergo oxidation to its disulfide at physiological pH in the presence of O₂. It has been suggested that this pro-oxidant activity of homocysteine is also responsible for the oxidation of LDL cholesterol and the damaging effect of homocysteine on vascular cells and tissues. This could be one of the mechanisms by which the coronary artery disease (CAD) occurs in patients with chronic renal failure (CRF).

In our study patients showed a significant decrease in non-enzymatic antioxidant levels, plasma glutathione, plasma thiols and serum albumin. The main role in the evolution of hypoproteinemia was played by albumin, while globulins were increased in this condition, hypoproteinemic stress cases were

characterized by a reduction of half-life, decrease in intra and extravascular albumin pools. The studies done in renal failure patients suggest serum albumin may be a major extracellular antioxidant in hemodialysis patients and that hemodialysis may prevent serum albumin reduction by inducing intermolecular sulfhydryl-disulfide exchange reaction which might also contribute to hypoalbuminemia. In plasma, free thiol groups which are the most important scavengers of hypochlorous acid are present as structural components of albumin. Chronic inflammatory stimuli result in phagocytic cell activation with subsequent increase in production of reactive oxygen species (ROS). Albumin via its thiol groups provides quantitatively 10 fold greater antioxidant protection against hypochlorous acid (HOCl)^[9].

Oxidation of non enzymatic antioxidant will decrease plasma antioxidant defenses and increase the likelihood of oxidant stress induced injury. Advanced oxidation protein products are proposed as one of the possible markers of oxidative injury, which originates under oxidative and carbonyl stress and increase inflammatory activity. Recently, the importance of advanced oxidation protein products has been pointed out as mediator monocyte activation state associated with chronic uremia. In our study there was no significant change in advanced oxidation protein products. In our study, the % haemolysis in chronic renal failure patients was found to be significantly increased in 0 hour and there was no significant increase in % hemolysis at 2 hours.

Red blood cells have a much shorter lifespan in the case of people with chronic renal failure than in the case of healthy subjects. The antioxidant defence system in red blood cells is impaired. The levels of glutathione and ascorbic acid are diminished, while the superoxide dismutase and glutathione peroxidase activities have been reported to be increased^[10].

In our study we found there was no significant change in total antioxidant activity. Average baseline levels of TBARS neither differ from those in controls nor rose during hemodialysis session. Malondialdehyde (MDA) is the easiest routine measure of lipid oxidation used by clinical laboratories. However, the determination of these aldehydes from measurements of thiobarbituric

acid reactive substance (TBARS) adducts clearly lacks specificity^[11].

In chronic renal failure there is decrease in serum iron due to loss of plasma proteins. Plasma protein levels are indicator of the inflammatory response. In chronic infections, -globulins are increased but the increase is smooth and wide based. In chronic renal failure due to kidney damage low molecular weight proteins are lost in urine (albumin, -1 antitrypsin) whereas high molecular weight plasma proteins are retained. In order to maintain the colloidal osmotic pressure during decreased albumin level the globulin fraction is increased^[9].

Several etiologic factors for oxidative stress have been proposed in these patients, including uremia, reaction of the blood with dialyzer membranes and subsequent contamination with dialysis byproducts, acute or chronic bacterial infections, parenteral administration of ferrous sulfate, limitation in consumption of fruits and vegetables (that are rich in antioxidant vitamins and natural phytochemicals) in the patients diet and underlying diseases. Oxidative stress in patients on hemodialysis is accompanied by several risks. It has been shown that it can lead to coronary artery disease (CAD), which is the most important cause of mortality and morbidity in ESRD patients^[12]. Furthermore, oxidative stress is responsible for alternations in the composition of lipoproteins that result in oxidation of low density lipoproteins and acceleration of atherosclerosis process. Finally, it seems that reactive oxygen species (ROS) has a role in the pathophysiology of ischemia-reperfusion injury. Due to the impact of ROS on the regulation of cellular cycle, oxygen radicals may cause hypertrophy of the renal tubular cells. It has been demonstrated that the formation of ROS has some effects on the development of kidney diseases such as glomerulonephritis, acute or progressive kidney failure, proteinuria and tubulointerstitial nephritis.

According to an earlier study conducted to assess the antioxidants levels and their relation with acute-phase reactants in patients on maintenance hemodialysis, there was no sex or age related differences in these factors. The only significant correlation was observed between the plasma level of glutathione, the C-reactive protein and serum albumin levels. While ESRD patients are susceptible to infections, the ROS formation is

a part of non-specific immune response against microorganisms. Reduction of the serum levels of these proteins such as albumin is suggestive of an inflammatory response. Thus, it is reasonable to see an association between acute-phase reactants and levels of antioxidants. To our knowledge, there is no study on such relations in hemodialysis patients.

However, no association has been found between these variables in other study populations. Bergesio and colleagues showed that measurement of total antioxidant capacity is not a reliable method for assessment of oxidative stress in patients with CKD^[13]. A recent study has suggested that antioxidant therapy may lessen cardiovascular complications in ESRD patients, suggesting that oxidant may be an important “nontraditional” factor^[14].

Conclusion

In our study the non-enzymatic antioxidant metabolites were found to be decrease in chronic renal failure. There was no significant increase in advanced oxidation protein products marker of protein oxidation in patients of chronic renal failure. There was a significant increase in % hemolysis the marker of membrane lipid damage at 0 hours sample whereas the 2 hour sample had shown no significant increase in patients with chronic renal failure. There was no significant increase in serum iron in patients of chronic renal failure.

It can be clearly concluded that there is oxidative stress in patients of chronic renal failure. We also observed that there was no significant decrease in total antioxidant activity in patients with chronic renal failure. This shows that the cellular damage may probably occur due to increased oxidative stress. We found a very highly significant hypoalbuminemia in patients with chronic renal failure. We also found significant hyperglobulinemia in patients with chronic renal failure.

This shows elevated inflammatory response in patients with chronic renal failure, which may be a defense response to prevent cellular damage. Further studies are needed to assess the effect of oxidative stress in different stages of renal failure. Major study will be to design effective antioxidant therapy and to analyze them in clinical studies, including mortality, to prove whether the concept holds true.

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