ORIGINAL ARTICLE

Role of Oxidant and Physiological Antioxidants in Progression of Chronic Renal Insufficiency

Shivaji Mane[1]

[1]*Department of Biochemistry, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai-400022, Maharashtra, India.*

Abstract:

Background: Oxidative stress plays a role in the pathogenesis of several diseases, including chronic renal insufficiency (CRI). There is considerable disequilibrium between oxidants and anti-oxidants in patients with CRI. Hence, the present study was undertaken to determine the role of oxidant and physiological antioxidants in progression of CRI. *Material and Methods:* The patients suffering from chronic renal failure (CRF) admitted in the medicine and Nephrology ward of Krishna Hospital Medical Research Center, Karad as well as out patients were included in the study. The diagnosis of renal disorder was done on the basis of clinical history, medical examination and laboratory investigations of urine and blood. The study was performed on 110 subjects; 66 were patients with CRI and 44 were normal healthy controls. *Results:* The mean values of serum creatinine, blood urea nitrogen and plasma lipid peroxide (LPO) were significantly elevated in CRI patients as compared to the controls and showed increasing trend from stage I to stage IV. The activities of erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes were found to be decreased in various stage of progressive renal failure. Also, it was found that level of transferrin was increased while albumin and ceruloplasm in values were decreased gradually in patients with progressive renal dysfunction. The plasma level of chain breaking antioxidant like ascorbic acid showed significant decrease with progression of renal failure while the bilirubin and uric acid levels were elevated in-patients with progressive renal failure. *Conclusion:* Free radicals are important mediators in many pathological and toxicological processes. Reduction in antioxidant capacity and increased lipid peroxidation probably plays a major role in progression of chronic renal failure.

Keywords: Oxidative stress; Chronic renal insufficiency; Oxidants; Antioxidants; Lipid peroxide; Superoxide dismutase; Progressive renal failure.

Introduction:

Chronic renal insufficiency ultimately culminating in end stage renal disease requiring dialysis or renal transplantation is a major health problem [1]. Numerous studies have examined the role of oxygen free radicals (OFR) in leukocyte dependent and independent models and the biological effects of these in glomerular pathophysiology [2, 3]. The partially reduced oxygen metabolites play an important role in the progression of renal disease to end stage renal disease (ESRD) in animal model [4-6].

Earlier studies have shown a significant imbalance in pro-oxidant and antioxidant activities in patients with renal dysfunction [7-9]. Furthermore, oxidative stress seems to increase as chronic kidney disease (CKD) progresses, CKD is a condition characterized by a gradual loss of kidney function over time. However, CKD progression correlates significantly and inversely with the level of glomerular filtration rate [10]. The majority of studies to-date have evaluated the grade of oxidative stress with a single biomarker, or a very limited number of them. Hence the present study was undertaken to determine the role of oxidant and physiological antioxidants in progression of CRI.

Material and Methods:

Total 66 patients aged between 22 to 68 years, suffering from CRI on the basis of clinical history and laboratory investigation and admitted in the medicine and nephrology ward of the Krishna Hospital Medical Research Center, Karad were included in the study. Age and sex matched 44 normal healthy controls from students and staffs of Krishna Institute of Medical Sciences Karad, having age group 20 to 60 years were

included in the study. The controls free from diseases such as diabetes mellitus, infection, hypertension, coronary artery disease, atherosclerosis and any renal dysfunction and no history of smoking were selected.

The patients with abnormal blood urea nitrogen and serum creatinine values were selected because these are standard biochemical markers for renal dysfunction. Patients with CRI were subdivided into various stages on the basis of degree of renal dysfunction as shown in Table 1. The serum Creatinine in a protein free filtrate was measured colorimetrically by Jaffe's reaction using Span Diagnostic Reagent Kits [11]. The Blood urea was measured colorimetrically by diacetyl monoxime method using Auto analyzer [12, 13].

The oxidative metabolism with reference to OFR was measured in the patients with CRI as well as in age and sex matched normal healthy controls. Plasma lipid peroxide (LPO) as the index of free radical activity was measured as thiobarbituric acid reactive substances (TBARS). The antioxygenic defense was measured consisting of erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Antioxidants in plasma such as transition metal binding protein Transferrin (TFR), ceruloplasmin (CER), albumin, ascorbic acid (AA), bilirubin and uric acid were also measured. The values of these parameters were processed. The data were treated by standard statistical methods to be given as mean ± SD.

Table No.1: Stages of chronic renal insufficiency

Stages	Serum Creatinine (mg%)	Blood Urea (mg%)
I	1.0-2.0	50-70
II	2.1-3.0	71-90
III	3.1-4.9	91-110
IV	>5.0	>111

Observations and Results:

The study comprised of 110 subjects of them, 66 were patients with CRI and 44 normal healthy controls. There was almost two-fold higher level of plasma LPO in the CRI patients while the enzymatic antioxidant defense in erythrocyte, activity of superoxide dismutase, catalase activity (CAT was expressed as mM of H_2O_2 decomposed/ mg Hb/min) and glutathione peroxidase (GPx) activity was significantly lower in progressive renal failure patients than controls. However, the plasma levels of transition metal chelating proteins (TFR, CER and Albumin) have shown changes in CRI patients when compared to normal control group. Transferrin level was very high in patients whereas ceruloplasmin and albumin levels were significantly lower in CRI patients. Plasma level of chain breaking antioxidants such as ascorbic acid, bilirubin and uric acid were significantly changed in CRI patients. Ascorbic acid was low whereas the plasma levels of bilirubin and uric acid were significantly elevated in patients with CRI as compared to control, (Table 2).

From the figure 1, it was observed that the values of serum creatinine, blood urea, and plasma lipid peroxide were significantly elevated as compared to the normal range of these analytes in controls and showed increasing trend from stage I to stage IV CRI. Increase in LPO with serum creatinine and urea (Figure 2) indicates that renal failure was closely related with lipid peroxidation.

The activities of erythrocyte SOD, CAT and GPx enzymes were found to be declining in various stage of progressive renal failure. The decline in the activity of catalase from stage I to stage II was very fast. After stage II, the decline was gradual. Also, table 3 indicates the relationship of progressive renal failure with prooxidant metal binding proteins (transferrin, ceruloplasmin and albumin) in plasma. It was found that level of transferrin increased with the progression of renal failure while albumin and ceruloplasmin values decreased gradually with the progression of renal dysfunction. The plasma level of chain breaking antioxidant like ascorbic acid (AA) is considered as the first line of defense against oxygen free radicals in aqueous phase. It showed significant decrease with progression of renal failure while the bilirubin and uric acid levels were elevated in-patients with progressive renal failure, (Table 3).

Table No. 2: Plasma LPO and erythrocyte SOD, CAT, GPx plasma, TFR, CER, Albumin, AA, BIL and Uric acid in patients with CRI and normal healthy control

Parameters	Normal controls		Patients with CRI	
	Mean ± SD	Range	Mean ± SD	Range
LPO nmols/ml	9.53 ±1.93	6.86-14.06	19.12±3.95	15.20-29.14
SOD unit /mg Hb	22.53±3.06	16.66-26.78	13.60±2.63	8.40-17.60
CAT (a)	543.82±69.67	465.76-823.92	346.26±63.58	224.76-432.80
GPxUnit/L	576.04±100.65	460.24-685.80	345.26±83.05	305.69-589.62
TFR mg/dL	204.81±38.69	142.00-321.18	560.96±85.48	390.20-807.00
CER mg/dL	38.47±6.87	26.65-52.90	22.86±4.89	12.90-29.95
Albumin gm/dL	3.94±0.49	3.00-4.50	3.36±0.53	2.50-4.50
Ascorbic Acid mg/dL	0.57±0.17	0.28-0.96	0.26±0.071	0.10-0.41
Bilirubin mg/dL	1.08±0.25	0.80-1.80	2.69±1.13	1.68-6.80
Uric Acid mg/dL.	3.16±0.64	1.60-4.62	5.24±1.07	4.00-6.90

Unit of catalase enzyme activity (a) = mM ofH2O2 decomposition / mg'Hemoglobin / min

Table No. 3: Relationship of progressive renal failure with enzymatic antioxidants, preventive antioxidants and chain breaking antioxidants

Antioxidants		Stage I, n=14	Stage II, n=19	Stage III, n= 16	Stage IV, n= 17
Enzymatic antioxidants (in erythrocytes)	SOD Unit/mgHb	15.29±2.22	14.00±1.80	13.26±2.62	11.35±2.35
	CAT (a)	377.70±62.0	312.20±70.19	295.27±65.81	294.22±67.18
	GPx Unit/L	401.12±83.14	397.67±79.62	369.29±89.17	312.24±92.20
Preventive antioxidants (in plasma)	Transferrin (mg/dL)	490.47±69.72	533.15±76.26	545.95±83.22	578.87±68.12
	Ceruloplasmin (mg/dL)	25.70±3.42	25.73±2.68	21.40±3.85	18.93±3.98
	Albumin gm/dL	3.72±0.56	3.66±0.62	3.70±0.67	3.26±0.44
Chain breaking antioxidants in plasma	Ascorbic acid mg/dL	0.36±0.05	0.30±0.07	0.26±0.08	0.23±0.04
	Bilirubin mg/dL	1.52±0.35	2.85±0.94	3.06±0.44	3.62±1.42
	Uric acid mg/dL	3.22±1.32	4.37±2.03	5.60±1.65	6.20±1.37

Unit of catalase enzyme activity (a) = mM ofH2O2 decomposition / mg'Hemoglobin / min

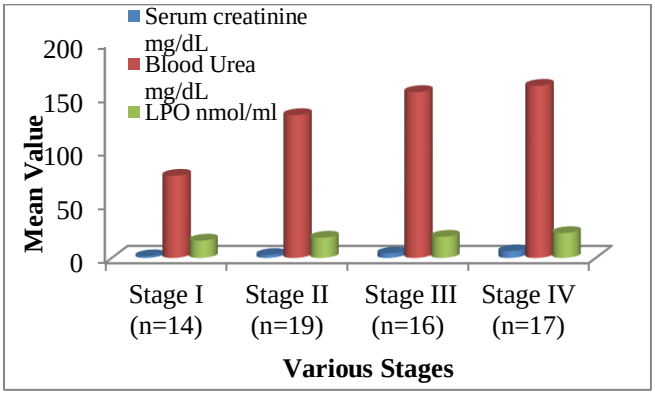

Figure 1: Relationship between Serum Creatinine, Blood Urea and Plasma LPO in various stages of CRF

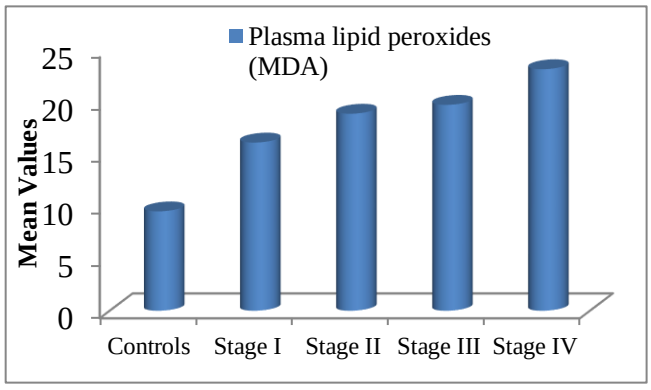

Figure 2: Plasma lipid peroxide level (MDA) in various stages of CRF and normal healthy controls

Discussion:

The renal failure is characterized by increase in serum creatinine and urea levels. It is known that in progressive renal failure glomerular filtration rate gradually decreases with simultaneous increase in serum creatinine and urea levels. Therefore, serum creatinine and urea is important biochemical marker for assessment of renal function. We studied the oxidative stress in patients with creatinimia and uremia. Patients with abnormal serum creatinine and urea showed significant increase in plasma LPO, a marker of oxidative stress. The degree of oxidative stress was found to increase with the progression of renal insufficiency. Oxidative stress was strongly correlated with serum creatinine and blood urea. Thus, OFR was found to play an important role in progression of chronic renal insufficiency. Growing data from experimental models support our hypothesis that oxidative stress boosts the progression-of primary renal disease to end stage renal disease (ESRD) of varied etiology. Possible causes for increased oxidative stress in, progressive chronic renal insufficiency:

1. Decreased capacity of natural antioxidants.
2. Hypertension in acute renal failure.
3. Change in xanthine dehydrogenase to xanthine oxidase in reperfusion injury.
4. Increased plasma free iron due to increased release from cell death and hemolysis (erythrocyte destruction).
5. Increased free radical activity due to nephrotoxic drugs.

Antioxidant defense of patients with progressive CKF (Chronic kidney failure) was found to be significantly decreased when compared with normal healthy controls. We studied antioxidants in blood, enzymatic, defense in erythrocyte and non-enzymatic antioxidants in plasma. The erythrocyte enzyme activities (SOD, CAT and GPx) were lowered in patients with progressive renal failure. Earlier studies [7, 14, and 15] have shown that superoxide dismutase is a free radical scavenger which has been recognized as a major defense against oxidizing effect of superoxide radical. The SOD steadily decreased with progression of renal failure. This is supported by previous reports [16, 17]. Although the mechanism involved in the decrease activity of erythrocyte, SOD is not clearly understood in CRF. It may be probably due to increase presence of OFR such as H_2O_2 which is known to suppress SOD activity, [18] altered gene expression and function of mitochondria in human nephritic syndrome. As results excess lipid peroxidation products were found in glomerular structures and subsequent damage at glomerular membrane causes proteinuria. Thus, mitochondrial dysfunction is a crucial pathophysiologic factor in proteinuria. The progressive renal failure showed significant decreasing trend in SOD activity. This resulted in decrease of superoxide radical scavenging capacity of renal tissues. Superoxide radicals participate in chain reactions to generate more oxidant free radicals. Plasma GPx activity is shown to be related to kidney function and is decreased in certain situations where nephrotoxic drugs are administered [17]. Patients with chronic renal failure often have a reduced level of blood selenium, decreased glutathione level and the activity of glutathione peroxidase and superoxide dismutase [19]. These evidences support the present results that erythrocyte GPx activities significantly decrease in the chronic renal insufficiency. This indicates decline of antioxidant defense mechanisms and results in increased H_2O_2 accumulation which can lead to increased free radical generation especially in presence of transition metal ions (Haber-Weiss and Fenton reactions). The decrease in GPx activity at the initial stage of CRF is very slow. From the mild renal insufficiency there was rapid decrease in the GPx activity and at the end stagerenal failure. GPx showed almost 50% activity in patients when compared controls. This lowering of activity of GPx could be due decreased GSH in chronic renal failure [20].

Catalase activity also declined fast in mild renal failure whereas in severe cases CRF, CAT activity remained unchanged because of increased H_2O_2 defender activity. Similarly, GPx slowly decreased in mild cases and fast in severe CRF. Erythrocyte CAT activity was significantly low in patients with chronic renal failure. Stage I had higher activity of catalase when compared to

the other stages. This may be due rapid decrease in GPx activity. H_2O_2accumulation in the erythrocyte causes higher activity of catalase to neutralize H_2O_2 into water and O_2. After stage I, the free radicals become dominant with progression of renal insufficiency. Therefore, a rapid decrease in the activity of catalase in stage III to stage IV was observed. Possible causes for decreased erythrocyte enzymatic antioxidant defense could be

1. Deficiency of trace elements such as Cu, Zn, Mn, Se or co-substrate (GSH) required for enzyme action.
2. Damage to structure of enzyme by free radicals or free radical activating factors.
3. Decreased expression of antioxidant gene by some factor in renal disease or DNA damage in intense oxidative stress of renal disease.

Present study investigated relationship between progressive renal failure and plasma levels of metal chelator proteins such as transferrin, ceruloplasmin and albumin. Transferrin is considered antioxidants because of its ability to bind with prooxidant Fe^{+3}. Free iron is responsible for OFR formation initiated by the reductive release of iron from transferrin. The transferrin level was derived indirectly from the plasma iron levels and which was significantly increased when compared to normal controls. The levels of transferrin remained higher with increasing severity of renal insufficiency. Our data are in contrast with previous studies [21-24] which demonstrated, that increased transferrin saturation capacity whereas there was no increase in apotransferrin level in renal failure. Our results indicated increased free iron level sin chronic renal insufficiency. The plasma iron levels are elevated due to distinctive hemolysis of erythrocytes as result of oxidative damage of erythrocyte membrane. Therefore, our study suggests that increased free iron concentration rather than transferrin is the basic mechanism in progression in End stage kidney disease (ESKD), since ceruloplasmin concentration was decreased in-patients with progressive renal failure. Low levels of ceruloplasmin fail to convert Fe^{+2} to Fe^{+3}. It is now known that free iron toxicodynamicis the chief mechanism for iron induced tissue damage, free radical production with resultant lipid peroxidation. Increased derived transferrin may be one of the most important causes for increased oxidative stress in parents with progressive renal failure. The concentration of transferrin was found to have increase whereas ceruloplasmin level was declining with progression of renal disease.

Major role of plasma albumin as chain breaking antioxidant is because of sulfhydryl (thiol) groups in the albumin molecule. In the present study, the albumin and ceruloplasmin levels were significantly decreased in patient with progressive renal failure. Previous report [25], support our hypothesis that albumin levels was declining because of oxidative injury in progressive renal insufficiency, which induces glomerular albumin leakage. Albumin being low molecular weight may have lost through glomerular basement membrane (GBM), due to changes in permeability in CRF or renal tubules may fail to reabsorb albumin and cause slight decrease in level of albumin through initial clinical "microalbuminuria". This is supported by the observation that a steady decrease in concentration of albumin occurs with progression chronic renal insufficiency. In our study, in various stages of CRF, patients showed decreasing trend from mild to severe chronic renal failure.

The present work reports a combination of increased plasma levels of endogenous antioxidant components such as uric acid, bilirubin and decreased plasma level of exogenous antioxidant vitamin ascorbic acid in plasma of patients with progressive CRF. These components are considered as part of body defense against free radical attack. Ascorbic acid is reduced in CRF patients probably due to high metabolic turnover rather than dietary deficiency or increased excretion via renal route. This could be related to free radical scavenging role of ascorbic acid in the state of increased free radical activity in progressive CRF. There was a close relationship between plasma ascorbic acid levels in various stages of CRF indicating that ascorbic acid is increasingly consumed in CRF is due to increased oxidative stress, Plasma bilirubin concentration was increased in CRF. There was no steady rise in bilirubin with progressive CRF. We hypothesized that increased bilirubin could be a consequence and not the cause of CRF. The rise in plasma bilirubin occurs as a result of

destruction of erythrocyte due to increased oxidative stress in CRF. Increased prevalence of anemia in CRF as reported by various studies and our unpublished work supports the hypothesis that increased oxidative stress across erythrocyte membrane and decreased antioxidant defense result in increased hemolysis. Subsequently plasma bilirubin levels rise noticeably in progressive renal failure.

Uric acid level was significantly ($P<0.001$) increased in patients with chronic renal insufficiency, when compared with normal healthy controls. Further, uric acid levels showed increasing trend with progression of renal failure. This increase in plasma uric acid level showed a good correlation with progression of renal failure. There could be mitochondrial dysfunction with increased dephosphorylation of ATP to AMP. AMP in turn must be catabolized to adenosine, hypoxanthine and finally uric acid by xanthine oxidase system. There could also be decreased uric acid excretion hi the progression of renal disease. Mitochondrial dysfunction is believed to occur in kidneys of patients with progressive renal failure with subsequent lipid peroxidation at the glomerular basement membrane. Thus, in renal failure the elevation of plasma uric acid occurs by this mechanism.

Thus, a vicious cycle must be working in a renal failure. Where at the initial start on point would be free radicals and then the magnification of free radical activity causes the biochemical deleterious reaction resulting in imbalance of metabolism in CRF, which is manifestation clinically in later stages of renal failure.

Conclusion:

We studied the oxidative stress in-patients with creatinemia and uremia. Patients with abnormal serum creatinine and urea showed significant increase in plasma LPO, a marker of oxidative stress. The degree of oxidative stress was found increased with progression of renal insufficiency. Oxidative stress was strongly correlated with serum creatinine and blood urea. Thus, OFR was found to plays.an important role in progression of chronic renal insufficiency. Growing data from experimental models supports our hypothesis that oxidative stress boosts the progression-of primary renal disease to end stage renal disease of varied etiology.

Conflict of Interest - Nil

Sources of Support - Nil

References

1. Vaidya SR, Aeddula NR. Chronic Renal Failure. In: StatPearls Publishing; 2021 January Treasure Island (FL): Available from: https://www.ncbi.nlm. nih.gov/ books/NBK535404/
2. Shah SV. Oxidants and iron in chronic kidney disease. *Kidney International* 2004;66, S50–55.
3. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxidants and Redox Signaling* 2014; 20 (7):1126-1167.
4. Carracedo J, Alique M, Vida C et al. Mechanisms of Cardiovascular Disorders in Patients with Chronic Kidney Disease: A Process Related to Accelerated Senescence. *Frontiers in Cell and Developmental Biology* 2020; 8 (Article185):1-14.
5. Chen SC, Huang JC, Su HM, Chiu YW, Chang JM, Hwang SJ et al. Prognostic cardiovascular markers in chronic kidney disease. *Kidney and Blood Pressure Research* 2018; 43:1388–1407.
6. Hasselwander O and Young IS. Oxidative stress in chronic renal failure. *Free Radical Research* 1998; 29(1):1–11.
7. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* 2010;4 (8):118-126.
8. Becker BN, Himmelfarb J, Henrich WL, Hakim RM. Reassessing the cardiac risk profile in chronic hemodialysis patients: a hypothesis on the role of oxidant stress and other non-traditional cardiac risk factors. *Journal of the American Society of Nephrology* 1997; 8(3): 475–486.
9. Tepel M, Echelmeyer M, Orie NN, Zidek W. Increased intracellular reactive oxygen species in patients with end-stage renal failure: effect of hemodialysis. *Kidney International* 2000; 58(2): 867–872.
10. Dounousi E, Papavasiliou E, Makedou A, Ioannou K, Katopodis KP, Tselepis A et al. Oxidative stress is progressively enhanced with advancing stages of CKD. *American Journal of Kidney Diseases* 2006; 48(5): 752–760.
11. Creatinine: Span Diagnostic Reagent. Kit Method Code no - 25921 Mfd. By Span Diagnosis Ltd.
12. Marsh WH, Fingerhut B, Miller H. Automated and

manual direct methods for the determination of blood urea. *Clinical Chemistry* 1965;11: 624-627.
13. Wybenga DR, Di Giorgio J, Pileggi VJ. Manual and automated methods for urea nitrogen measurement in whole serum. *Clinical Chemistry* 1971; 17(9):891-895.
14. Rosa AC, Corsi D, Cavi N; Bruni N, Dosio F. Superoxide Dismutase Administration: A Review of Proposed Human Uses. *Molecules* 2021; 26:1844.
15. Ighodaro OM and Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. *Alexandria Journal of Medicine* 2018; 54(4):287-293.
16. Tan RJ, Zhou D, Xiao L, et al. Extracellular Superoxide Dismutase Protects against Proteinuric Kidney Disease. *Journal of American Society of Nephrology* 2015; 26(10):2447-2459.
17. Whitin JC, Tham DM, BhamreS, OrntDB, Scandling JD, Tune BM, Salvatierra O, Avissar N and Cohen HJ. Plasma glutathione peroxidase and its relationship to renal proximal tubule function. *Molecular Genetics and Metabolism* 1998; 65 (3):238 - 245.
18. Kono, Y. and Fridovich I. Superoxide radical inhibits catalase. *Journal of Biological Chemistry* 1982; 257:5751–5755.
19. Schettler V, Wieland E, Methe H et al. Oxidative stress during dialysis: effect on free radical scavenging enzyme (FRSE) activities and glutathione (GSH) concentration in granulocytes. *Nephrology Dialysis and Transplantation* 1998;13 (10):2588–2593.
20. Nadkarni GD, Sawant BU. Role of antioxidant defence in renal pathophysiology: primacy of glutathione peroxidase. *Indian Journal of Experimental Biology* 1998;36 (6):615-617.
21. Gaweda AE. Markers of iron status in chronic kidney disease. *Hemodialysis International* 2017; 21(1):S21-S27.
22. Macdougall IC. Iron supplementation in the non-dialysis chronic kidney disease (ND-CKD) patient: oral or intravenous? *Current Medical Research and Opinion* 2010; 26 (2): 473–482.
23. Fishbane S, Kalantar-Zadeh K, Nissenson AR. Serum ferritin in chronic kidney disease: reconsidering the upper limit for iron treatment. *Seminars in Dialysis* 2004; 17 (5): 336–341.
24. Boam J, Mikkail MS, Pargraudeau PH, Palau PR, Rommey SL. *American Journal of Obstetrics and Gynecology* 1990;163: 1949-1952.
25. Cheung PK, Baller JF, Bakker WW. Impairment of endothelial and subendothelial sites by a circulating plasma factor associated with minimal change disease. *Nephrology Dialysis Transplantation* 1996; 11(11):2185-2191.

Address for correspondence: *Dr. Shivaji Mane, Additional professor, Department of Biochemistry, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai- 400022, Maharashtra, India.*
Email:maneshivajid64@gmail.com,
Mobile: +91 9969419325.

How to cite this article: Shivaji Mane. Role of Oxidant and Physiological Antioxidants in Progression of Chronic Renal Insufficiency. Walawalkar International Medical Journal 2021; 8(1):82-88. http://www.wimjournal.com

Received date: 27/07/2021 Revised date: 01/09/2021 Accepted date: 01/09/2021