

Oxidant-Antioxidant Balance in Uremic Patients with CKD and on Renal Replacement TherapySrilakshmi Katari¹, Sowjanya Yerram^{2*}, Kalavathi Chanamallu³, Bhanu Kumar Chitteti⁴, Sobha Devi Kolla⁵, Nissi Namratha Thathapudi⁶^{1,2}Assistant Professor, Department of Biochemistry, Guntur Medical College, Guntur³Professor, Department of Biochemistry, Government Medical College, Nandyal⁴Assistant Professor, Department of Biochemistry, Guntur Medical College, Guntur⁵Professor & HOD, Department of Biochemistry, Guntur Medical College, Guntur⁶Post Graduate 2nd Year, Department of Biochemistry, Guntur Medical College, Guntur

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Conflict of interest: Nil

Abstract:**Background:** Oxidative stress is the main underlying mechanism in CKD patients, leading to cardiovascular disease and renal failure. The antioxidant response to the oxidative stress, is however altered with the renal replacement therapies (RRT) like Hemodialysis (HD) and Peritoneal Dialysis (PD) and also obviously by the intensity of damage to the kidneys (Stage of CKD-based on eGFR).**Aim and Objectives:** The current study aims to evaluate the homeostatic response of the body to the oxidative damage by the ROS.**Methods:** Estimation of pro-oxidant status by assay of thiobarbituric acid reactive substances, hydroperoxides and protein carbonyls. The defensive antioxidant substances like, SOD, Catalase, GSH peroxidase, GSH reductase were studied.**Results:** TBARS and LPO were found high in patients undergoing Hemodialysis and protein carbonyls were high in Peritoneal dialysis patients. The antioxidants were declined in severe stage of CKD and also in HD patients.**Conclusion:** Our study showed that impaired renal function and duration of dialysis treatment are associated with increased Oxidative stress condition. Further investigation is still required in order to estimate the imbalance between antioxidant and oxidant factors.**Keywords :** Chronic Kidney Disease(CKD), Renal Replacement Therapy(RRT), Hemodialysis(HD), Peritoneal Dialysis(PD), Glomerular Filtration Rate(GFR), End-Stage Renal Disease(ESRD), Reactive Oxygen Species(ROS), Oxidative Stress (OS), Thio Barbituric Acid Reactive Substances(TBARS).This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Oxidative stress is the main underlying mechanism in CKD patients, leading ultimately to cardiovascular disease and renal failure [1]. Uremic patients have both traditional cardiovascular (CV) risk factors (old age, hypertension, diabetes, smoking, dyslipidemia, heart failure, and physical inactivity) and non-traditional CV risk factors including malnutrition, anemia, hyperhomocysteinemia, neuropathy, hyperparathyroidism, and chronic inflammation [2-4].

Patients with end-stage renal disease (ESRD) undergoing renal replacement therapy (RRT), either hemodialysis (HD) or peritoneal dialysis (PD), may face a partial loss of some low-molecular-weight plasma factors (i.e., vitamins A, C, and E)[5,6] that normally contrast inflammation

by neutralizing reactive oxygen species (ROS). The imbalance in antioxidant and pro-oxidant factors generates an oxidative stress (OS) that increases the inflammatory state already present in these patients. OS results from an imbalance between prooxidant and antioxidant defence and mechanisms with increased levels of prooxidant leading to tissue damage [7].

Antioxidants can be divided into intracellular and extracellular antioxidants. Intracellular enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-GPx), which convert substrates (superoxide anion radicals and hydrogen peroxide) to less reactive forms [7]. Various extracellular antioxidants, such as reduced glutathione (GSH), bilirubin, uric acid, and iron (Fe), prevent free radical (FR) reaction by

sequestering transition metal ions by chelation in plasma.

Many studies have demonstrated increased OS in patients with CKD, including accumulation of reactive carbonyl compounds as markers of elevated protein peroxidation[8,9] and increased concentrations of thiobarbituric acid reactive substances(TBARS), malondialdehyde(MDA), and Hydroperoxides(LPO) as markers of high lipid peroxidation[10,11]. OS is particularly detrimental in patients receiving HD after each dialysis session, due to the contact of blood with dialysis membrane, facing a chronic deficit in antioxidant defense system. Thus, this study was undertaken in order to determine the effect of different stages of CKD, hemodialysis, and peritoneal dialysis on lipid peroxidation, protein oxidation, and antioxidant defence.

Aim and Objectives:

1. To study the effect of Chronic Kidney Disease CKD on the balance between oxidant and anti-

oxidant mechanisms in the pathogenesis of the disease course.

2. To study the effect of renal replacement therapies-Hemodialysis and Peritoneal dialysis on the balance between oxidant and antioxidant mechanisms.

Materials & Methods:

The study was carried out in 156 patients with CKD. Patients were divided into **6 groups** according to the classification of the **National Kidney Foundation (NKF)**.

The six groups include:

1. 25 patients with GFR equal to 87.32 mL/min (CKD 1),
2. 25 patients with GFR equal to 48 mL/min (CKD 2),
3. 25 patients with GFR equal to 21.22 mL/min (CKD 3),
4. 18 patients with GFR equal to 11.11 mL/min (CKD 4),
5. 40 hemodialysis (HD) patients, and
6. 23 peritoneal dialysis (PD) patients.

Table 1:

	CKD 1 n = 25	CKD 2 n = 25	CKD 3 n = 25	CKD 4 n = 18	HD n = 40	PD n = 23
Age (years)	38 ± 15	52 ± 14	47 ± 13	43 ± 13	40 ± 18	40 ± 12
Weight (kg)	70 ± 12	69 ± 15.32	61 ± 17	59 ± 13.33	58 ± 18.77	66.7 ± 19.1
BMI (Kg/m ²)	25.07 ± 10.0	26.22 ± 6.12	23.9 ± 4.21	24.88 ± 5.1	24.11 ± 4.02	26.19 ± 3.33
Sex ratio (M/F)	12/13	18/7	16/9	11/7	27/13	17/6
GFR (ml/min)	87.32 ± 22.77	48 ± 7.12	21.22 ± 4.89	11.11 ± 3.83	—	—

Exclusion Criteria:

- Clinical signs of infection (hepatitis B and hepatitis C)
- Malignancy
- Diabetes
- Nephrotic syndrome
- Active immunological diseases
- Immunosuppressive or immuno modulatory
- Anti-inflammatory drugs

We calculate the creatinine clearance from the serum creatinine and through the following estimation formula: the Cockcroft and Gault formula [GFR (1/4) (140 – age) BW × 1.23/creatinine]. In women, this value was multiplied by 0.85. Hemodialysis HD patients were on standard bicarbonate using polysulfone membrane.

Patients were dialyzed from 09 to 86 months, three times a week, and each session lasting 4 hours. Peritoneal dialysis PD patients were in dialysis from 06 to 62 months, using a standard procedure (four exchanges: three isotonic 1.36% glucose solutions, then a hypertonic one at 3.86% glucose). We note that the patients' nutrition contains small amounts of protein and phosphate.

Assays: In all the patients, blood samples were drawn after 12-hour overnight fast from antecubital vein in uremic and PD patients and from the dialysis fistula in HD patients. Vacutainer tubes with different anticoagulants during the blood samples are used. Plain vacutainers tubes are used for biochemical analysis, and those containing EDTA are used for the hematology analysis. We collected serum, with low speed centrifugation at 3000 ×g at 4°C for 15 min. The serum was removed, aliquoted and stored at –20°C.

Lipid and Protein Peroxidation analytes: By measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides (LPO). TBARS concentrations were measured according to the method of Quintanilha et al., using tetramethoxypropane (Prolabo) as precursor of malondialdehyde (MDA). One milliliter of diluted plasma (protein concentration about 2 mg/mL) was added to 2 mL of thiobarbituric acid (final concentration, 0.017 mmol/L) and butylated hydroxytoluene (concentration, 3.36 mmol/L) and incubated for 15 min at 100°C. After cooling and centrifugation, the absorbance of supernatant was

measured at 535 nm. Data were expressed as mmol of TBARS produced/mL of sample.

The plasma was also assayed to determine LPO with an assay kit from the company Cayman Chemicals; the rate of increase in the absorbance at 500 nm is directly proportional to the concentration of LPO produced. Oxidized proteins were estimated by measuring carbonyls concentrations. The latter were analyzed in plasma with an assay kit from Cayman Chemicals company kit; the absorbance of the samples was measured between 360 and 385 nm by a plate reader.

Antioxidant Measurements: Activity of antioxidant enzymes was measured in erythrocyte. Superoxide dismutase (SOD) activity was determined with Sigma Chemical kits at 450 nm by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit was defined as the amount of enzymes necessary to produce 50% inhibition in the rate of p-iodonitrotetrazolium reduction. Catalase (CAT) activity was measured with an assay kit from Cayman Chemical company kit. CAT is involved in the detoxification of hydrogen peroxide (H₂O₂). CAT enzyme activity could be determined by using the peroxidatic function of CAT at 540 nm.

Glutathione peroxidase (GSH-Px) enzyme activity was measured with an assay kit from Sigma Chemical company kit. GSH-Px activity was measured indirectly by a coupled reaction with glutathione reductase (GSH-GR). Oxidized glutathione

(GSSG), produced upon reduction of an organic hydroperoxide by GSH-Px, was recycled to its reduced state by GSH-GR and NADPH. The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm and is indicative of GSH-GPx activity. Glutathione reductase (GSH-GR) enzyme activity was determined with an assay kit from Sigma Aldrich Chemicals company kit. GSH-GR catalyzed the reduction of GSSG to reduced glutathione (GSH). GSH-GR activity can be measured either by the decrease in absorbance caused by the oxidation of NADPH at 340 nm or by the increase in absorbance caused by the reduction of DTNB at 412 nm.

Statistical Analysis: Statistical analysis was performed using SPSS 20.0 (IBM SPSS statistics; USA).

Data were expressed as the mean \pm SD (standard deviation). The distribution of variables was compared by the χ^2 analysis. Difference between the arithmetical averages was assessed by ANOVA, which was adjusted for multiple comparisons.

Depending on the normality of distribution of variables, the comparisons between groups were performed using one-way of analysis of variance (ANOVA) or the Mann-Whitney U-test when results are non-parametrically distributed. All statistical tests were two tailed, and a P value below 0.05 was considered statistically significant.

Results:

Table 2:

	CKD 1 n = 25	CKD 2 n = 25	CKD 3 n = 25	CKD 4 n = 18	HD n = 40	PD n = 23
Age (years)	38 \pm 15	52 \pm 14	47 \pm 13	43 \pm 13	40 \pm 18	40 \pm 12
Weight (kg)	70 \pm 12	69 \pm 15.32	61 \pm 17	59 \pm 13.33	58 \pm 18.77	66.7 \pm 19.1
BMI (Kg/m ²)	25.07 \pm 10.0	26.22 \pm 6.12	23.9 \pm 4.21	24.88 \pm 5.1	24.11 \pm 4.02	26.19 \pm 3.33
Sex ratio (M/F)	12/13	18/7	16/9	11/7	27/13	17/6
GFR (mL/min)	87.32 \pm 22.77	48 \pm 7.12	21.22 \pm 4.89	11.11 \pm 3.83	—	—
Dialysis duration (months)	—	—	—	—	09 to 86 months	06 to 62 months
Urea (g/L)	0.44 \pm 0.12	0.60 \pm 0.21	1.11 \pm 0.42	1.71 \pm 0.56	1.29 \pm 0.36	1.10 \pm 0.28
Creatinine (mg/L)	9.88 \pm 3.1	17.89 \pm 6.10	40.28 \pm 12.12	73.56 \pm 12.01	103 \pm 32.01	71 \pm 39.88
Uric acid (g/L)	51.22 \pm 13.01	69 \pm 11.02	83.21 \pm 13.98	82.65 \pm 19.98	63 \pm 12.78	65.78 \pm 14.88
Total proteins (g/L)	74.99 \pm 4.87	73.28 \pm 6.55	71.24 \pm 14.00	74.74 \pm 9.56	38 \pm 8.77	66.54 \pm 7.56
Cholesterol (mg/dL)	170 \pm 33.5	190 \pm 24.1	178 \pm 32.5	180 \pm 45.5	230 \pm 158	200 \pm 59
HDL-cholesterol (mg/dL)	57 \pm 11	46 \pm 10	51 \pm 11	48 \pm 16	39 \pm 17	48 \pm 9
LDL-cholesterol (mg/dL)	96 \pm 38	120 \pm 39	96 \pm 88	99 \pm 39.5	124 \pm 57	129 \pm 48
Triglycerides (mg/dL)	121 \pm 57	143 \pm 53.8	151 \pm 76	138 \pm 59	171 \pm 90	179 \pm 91
Erythrocytes,	4.5 \pm 0.69	4.7 \pm 0.59	3.98 \pm 0.75	3.1 \pm 0.39	3.10 \pm 0.88	3.0 \pm 0.56

×106/μL						
Hemoglobin (g/dL)	12.9±1.44	12.3±1.22	11.5±1.9	7.8±1.11	8.9±1.6	9.88±2.1

Oxidative Status assessment: Results showed that the levels of lipid oxidative product (TBARS and LPO) and protein carbonyls were significantly increased in HD and PD patients compared to the other stages of CKD (P < 0.001) The levels were compared with CKD stage 1 patients.

TBARS concentrations were 2.8-fold higher in HD, 2.3-fold higher in PD, and 1.7-fold higher in CKD4 than in CKD1 patients (P < 0.001).

LPO were also 6-fold higher in HD, 4.1-fold higher in PD, and 5.2-fold higher in CKD4 than in CKD1 patients (P < 0.001).

Carbonyls were 3-fold higher in HD, 3.5-fold higher in PD and 2.2-fold higher in CKD4 than in CKD1 patients (P < 0.001). Our results showed a considerable increase on protein carbonyls in PD patients. The levels of the latter products were increased at stage 3 and stage 4 of CKD samples.

Oxidative status in the study groups

Table 3:

	CKD 1	CKD 2	CKD 3	CKD 4	HD	PD	P
TBARS (μmol/L)	0.67 ± 0.16	0.71 ± 0.10	0.81 ± 0.11	1.139 ± 0.36	1.876 ± 0.16	1.541 ± 0.26	<0.001
LPO (nmol/mL)	0.42 ± 0.29	0.98 ± 0.33	1.19 ± 0.38	2.184 ± 0.16	2.52 ± 0.36	1.722 ± 0.22	<0.001
Carbonyls (nmol/mg)	0.68 ± 0.19	0.97 ± 0.17	1.11 ± 0.37	1.496 ± 0.29	2.04 ± 0.14	2.38 ± 0.15	<0.001

Statistically significant differences between all the groups (P < 0.001).

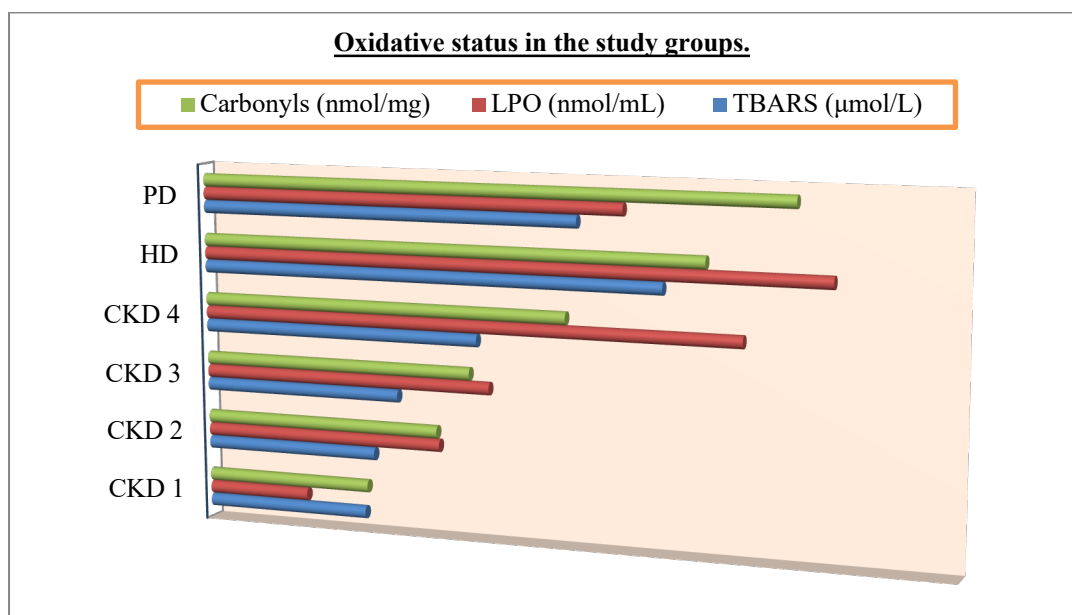


Figure 1:

Antioxidative Status assessment:

The levels were compared with CKD stage 1 patients. Our results showed that SOD activity was decreased by 68% in CKD4, 63% in HD and by 65% in PD than in CKD1 patients (P < 0.001).

CAT activity was also significantly decreased by 81% in CKD4, 78% in HD and by 84% in PD than

in CKD1 (P < 0.001). A decrease in GSH-Px activity was noted in HD by 69% in CKD4, 54% in HD and by 65% in PD than in CKD1 (P < 0.001).

Similar results were observed for GSH-GR activity, which diminished by 63% in CKD4, 59% in HD and by 69% in PD than in CKD1 (P < 0.001).

Antioxidative status in the study groups

Table 4:

	CKD 1	CKD 2	CKD 3	CKD 4	HD	PD	P
SOD (U/mL)	82.31 ± 8.11	65.84 ± 2.22	68.31 ± 7.10	55.97± 2.88	51.85 ± 3.38	53.50 ± 4.32	<0.001
CAT (U/mL)	64.44 ± 5.28	56.70 ± 7.38	56.70 ± 8.21	52.19 ± 3.33	50.26 ± 5.38	54.12± 5.81	<0.001
GSH-Px (U/mL)	7.9 ± 1.38	6.63± 0.38	5.84± 0.88	5.45 ± 0.87	4.26 ± 1.44	5.13 ± 1.20	<0.001
GSH-GR (U/mL)	1.8 ± 0.09	1.54± 0.17	1.29± 0.17	1.13± 0.22	1.06± 0.15	1.24± 0.19	<0.001

Statistically significant differences between all the groups ($P < 0.001$).

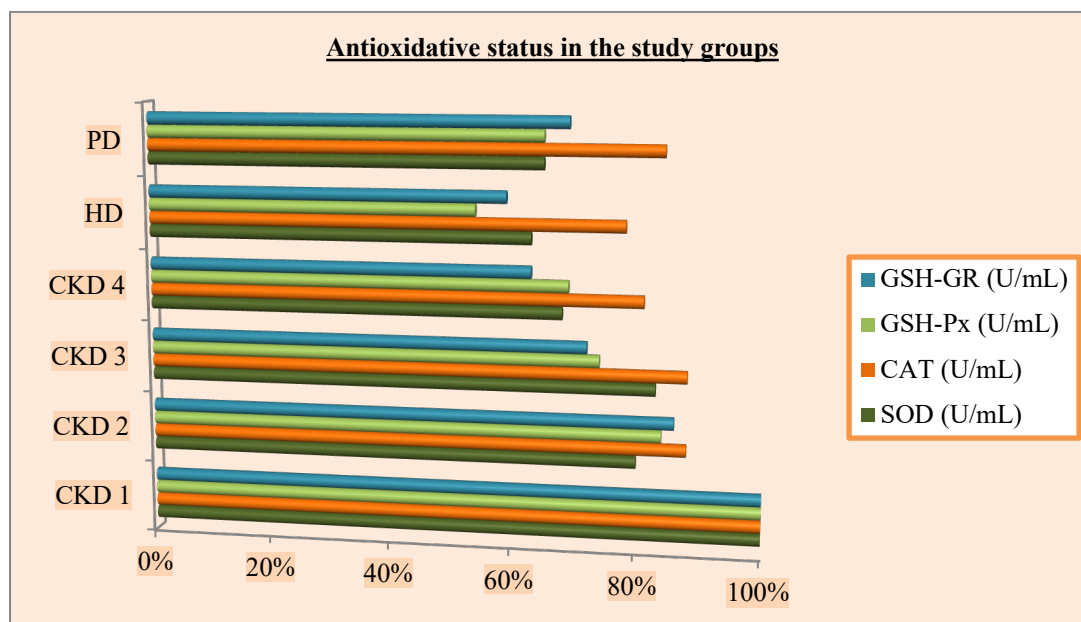


Figure 2:

Discussion

The results suggest that CKD patients have increased oxidative stress produced by an imbalance between pro- and antioxidant mechanisms [12,13]. This oxidative stress is responsible for the peroxidation of macromolecules such as lipids and proteins causing significant damage.

Several pathophysiologic mechanisms have been suggested; some studies suggest that due to low availability of "thiol" in malnutrition and hypoalbuminemia conditions and in other cases to "uremic status" itself with solute retention, favouring their pathogenicity; and also due to the association of comorbid factors such as advanced age, diabetes, and inflammatory and infectious phenomena [14,15]. Also, when the uremic patients receive treatment, as in HD, with extra renal techniques, oxidative stress is promoted by several reasons among which the usage of low biocompatible synthetic membranes and the lack of ultrapure dialysis water stand out mechanisms [16].

Oxidative stress present in the CKD patients, favours the precocious atherosclerosis and ultimately to cardiovascular disease in these patients. Moreover, the effect of different RRT on oxidative status compared to unanalysed patients with different stage of CKD has not been clearly described. In the study, we found an increase in markers of lipid peroxidation, in which a production of TBARS and LPO and production of protein oxidation was significantly higher already at severe stages of CKD. Carbonyls were increased at severe stages of CKD and its production was increased in both HD and PD patients. Our results were in agreement with previous findings which associated the increased levels of lipid peroxidation with the severity of coronary atherosclerosis in patients with CKD[17].

Free radicals derived from oxygen are the source of lipid peroxidation, and the first line of defence against them is SOD. Its function is to catalyse the conversion of superoxide radicals to hydrogen peroxide (H₂O₂). Indeed, the decreased SOD activity in our study, especially in HD patients, suggests that accumulation of superoxide anion

radical might be responsible for increased lipid peroxidation [18].

Glutathione is a tripeptidic thiol found in the inside the cells and is the most important cellular antioxidant. Oxidized glutathione (GSSG) is highly toxic to cells so that the organism tends to reduce GSSG to GSH through glutathione reductase. Thus, determining GSH-GR is considered a reliable estimate of the degree of cellular oxidative stress [19].

Moreover, GSH-Peroxidase is responsible for the most decomposition of lipid peroxide and protects the cell from the deleterious effects of peroxides. H₂O₂ in the presence of sufficient CAT activity will be converted to harmless H₂O and O₂. The patients have decreased levels of the four enzymes; this decrease was more important in HD and PD patients. Hence our result explained the reason of the increase in TBARS and LPO levels.

Moreover, in the Hemodialysis population, the interaction between dialysis membranes and blood can trigger the release of oxygen-free radicals and oxidizing agents, such as superoxide anion, hydrogen peroxide, and myeloperoxidase. In turn, these molecules contribute to the oxidation of lipid products, proteins and nucleic acids. This oxidation has several pathophysiological consequences, including enhanced atherogenicity of Ox-LDL, and accelerated demise of circulating erythrocytes, leading to a shorter life span. The patients were treated by a conventional peritoneal dialysis, which is considered as bio incompatible.

Repeated and long-term exposure to conventional glucose-based peritoneal dialysis fluids plays a central role in the pathogenesis of the functional and structural changes of peritoneal membrane. Low pH, high glucose concentration, high osmolality and heat sterilization represent the major factors for the low biocompatibility [20].

Bio incompatibility of peritoneal dialysis solutions seems to play a central role in the increase of ROS production [21]. Consequently decreased antioxidants defence mechanisms, which leads to promote the malnutrition, inflammation and atherosclerosis.

Conclusion

Our study showed that impaired renal function and duration of dialysis treatment are associated with increased Oxidative stress condition. Further investigation is still required in order to estimate the imbalance between antioxidant and oxidant factors, and in particular it is essential to investigate whether different RRT achieve similar levels of OS, especially in patients with high risk to develop CVD.

Our data suggest that patients subjected to RRT need new methods aimed to reduce intradialytic oxidative stress, such as like incorporating antioxidant therapy into the dialysis membrane, hemolipodialysis, using of electrolyte-reduced water for dialysate or using an ultrapure dialysate system to reduce acute phase inflammation.

References

1. Schiffrin EL, Lipman ML, Mann JFE. Chronic kidney disease: effects on the cardiovascular system. *Circulation*. 2007;116(1):85–97.
2. Q. Yao, J. Axelsson, P. Stenvinkel, and B. Lindholm, "Chronic systemic inflammation in dialysis patients: an update on causes and consequences," *ASAIO Journal*, 2004; 50(6): 13–17.
3. Y. Nishizawa and H. Koyama, "Endogenous secretory receptor for advanced glycation end-products and cardiovascular disease in end-stage renal disease," *Journal of Renal Nutrition*, 2008; 18(1): 76–82.
4. H. F. Tbahriti, D. Mekkassi, R. Moussaoui et al., "Inflammatory status in chronic renal failure: the role of homocysteinemia and pro-inflammatory cytokines," *World Journal of Nephrology*, 2013; 2(2): 31–37.
5. Lim P, Chan E, Lu T, et al. Lipophilic antioxidants and iron status in ESRD patients on hemodialysis. *Nephron*. 2000;86(4):428–435.
6. Uzum A, Toprak O, Gumustas MK, Ciftci S, Sen S. Effect of vitamin E therapy on oxidative stress and erythrocyte osmotic fragility in patients on peritoneal dialysis and hemodialysis. *Journal of Nephrology*. 2006;19(6):739–745.
7. M. Annuk, M. Zilmer, and B. Fellström, "Endothelium-dependent vasodilation and oxidative stress in chronic renal failure: Impact on cardiovascular disease," *Kidney International, Supplement*, 2003; 63: S50–S53.
8. Locatelli F, Canaud B, Eckardt K, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging treat to patient outcome. *Nephrology Dialysis Transplantation*. 2003;18(7):1272–1280.
9. Lucchi L, Bergamini S, Iannone A, et al. Erythrocyte susceptibility to oxidative stress in chronic renal failure patients under different substitutive treatments. *Artificial Organs*. 2005; 29(1):67–72.
10. R. Ramos and A. Martínez-Castelao, "Lipoperoxidation and hemodialysis," *Metabolism*, 2008; 57(10): 1369–1374.
11. D. R. Suresh, S. Delphine, and R. Agarwal, "Biochemical markers of oxidative stress in predialytic chronic renal failure patients," *Hong Kong Journal of Nephrology*, 2008; 10(2): 69–73.
12. M. Dirican, E. Sarandol, Z. Serdar, N. Ocak, and K. Dilek, "Oxidative status and prevalent

- cardiovascular disease in patients with chronic renal failure treated by hemodialysis," *Clinical Nephrology*, 2007; 68(3): 144–150.
13. M. González Rico, M. J. Puchades, R. García Ramón, G. Saez, M. C. Tormos, and A. Miguel, "Effect of oxidative stress in patients with chronic renal failure," *Nefrologia*, 2006; 26(2): 218–225.
 14. T. Miyata, K. Kurokawa, and C. de Strihou, "Relevance of oxidative and carbonyl stress to long-term uremic complications," *Kidney International, Supplement*, 2000; 76: S120–S125.
 15. C. Maritim, R. A. Sanders, and J. B. Watkins, "Diabetes, oxidative stress, and antioxidants: a review," *Journal of Biochemical and Molecular Toxicology*, 2003; 17: 24–38.
 16. Guo, C. Wang, P. Chen, and T. Yang, "Linkage of some trace elements, peripheral blood lymphocytes, inflammation, and oxidative stress in patients undergoing either hemodialysis or peritoneal dialysis," *Peritoneal Dialysis International*, 2011; 31(5): 583–591.
 17. C. H. Guo, W. S. Ko, P. C. Chen, G. S. Hsu, C. Y. Lin, and C. L. Wang, "Alterations of trace elements and oxidative stress in uremic patients with dementia," *Biological Trace Element Research*, 2009; 131: 13–24.
 18. B. Cestaro, A. Giuliani, F. Fabris, and C. Scarafioti, "Free radicals, atherosclerosis, ageing and related dysmetabolic pathologies: biochemical and molecular aspects," *European Journal of Cancer Prevention*, 1997; 6, supplement 1: S25–S30.
 19. J. Valentini, G. C. Schmitt, D. Grotto et al., "Human erythrocyte δ -aminolevulinate dehydratase activity and oxidative stress in hemodialysis patients," *Clinical Biochemistry*, 2007; 40:9-10: 591–594.
 20. S. Müller-Krebs, L. P. Kihm, B. Zeier et al., "Glucose degradation products result in cardiovascular toxicity in a rat model of renal failure," *Peritoneal Dialysis International*, 2010; 30(1): 35–40.
 21. D. C. Tarnag, T. W. Chen, T. P. Huang, C. L. Chen, T. Y. Liu, and Y. H. Wei, "Increased oxidative damage to peripheral blood leukocyte DNA in chronic peritoneal dialysis patients," *Journal of the American Society of Nephrology*, 2002; 13: 1321–1330.