

Evaluation of oxidative stress association with chronic kidney disease

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Abstract: The present study was aimed to investigate the correlation of oxidative stress with renal failure in Chronic Kidney Disease (CKD) patients. Oxidative stress was measured via superoxide dismutase (SOD), Glutathione (GSH), Malondialdehyde (MDA) and catalase assessment. For renal dysfunction, basic renal parameters, urea, creatinine and uric acid were determined. 25 patients of moderate CKD and 25 patients of severe CKD were selected for the present study as case group along with 25 normal individuals as control group. Oxidative stress parameters (SOD, GSH, MDA and catalase) were assessed by spectrophotometric assay while renal parameters (urea, creatinine and uric acid) were estimated by enzymatic kit method. We concluded that the decrease in renal GSH in chronic kidney disease could be explained by its consumption in scavenging free radicals and maintaining the redox state of the cell during CKD. Significant increase in both blood urea nitrogen (BUN) and creatinine showed that CKD resulted in serious renal injury.

[Zaigham K, MT Muneer, MS Iqbal, MN Hafeez and Q Ali. **Evaluation of oxidative stress with chronic kidney disease.** *N Y Sci J* 2015;8(3):88-92]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 15

Keywords: Chronic Kidney Disease (CKD), Oxidative stress, catalase, redox, creatinine

Introduction

Renal failure or kidney failure formerly called renal insufficiency which describes a medical condition in which kidney fails to adequately filter toxin and waste product from the blood [1]. Chronic renal failure (CRF) effects the kidney more slowly. CRF may be due to primary kidney diseases such as glomerular nephritis and renal artery stenosis, diabetes or hypertension. If left untreated, kidney function will further deteriorates as indicated by a fall in glomerular filtration rate and finally End Stage Renal Failure ESRF [2].

Chronic renal failure (CRF) has numerous causes; the most common is diabetes mellitus. The second most common is long-standing, uncontrolled hypertension or high blood pressure. Polycystic kidney disease is another well-known cause of CRF. The majority of people afflicted with polycystic kidney disease have a family history of the disease. Other genetic illnesses affect kidney function as well. Over use of common drugs such as aspirin, Ibuprofen and acetaminophen (paracetamol) can also cause chronic kidney damage [3]. A progressive renal failure gives rise to steady increase in parathyroid hormone. After year of a chronic renal failure secondary hyperparathyroidism would increase in bone turn over leading to elevated serum alkaline phosphatase [4]. There is considerable disequilibrium between oxidants and anti-oxidants in patients with chronic renal insufficiency (CRI). Evidence suggests that ROS are not merely the consequence of treatment or progress of the disease but one of the

causal agents of CRI, and that oxidative stress (OS) can take place even in the absence of hemodialysis. Patients with uremia have diminished response to OS due, probably, to a decrease in the antioxidant capacity; the mechanisms underlying this decrease, however, are not well established [5].

Recently, it was suggested that patients with CKD are exposed to other nontraditional uremia-related risk factors, such as anemia, altered calcium-phosphorus metabolism, inflammation, malnutrition, and oxidative stress [6]. Oxidative stress takes place when oxidant production exceeds local antioxidant capacity, resulting in increased oxidation of important macromolecules, including proteins, lipids, carbohydrates, and damage of DNA structure. Oxidative stress was proposed to have a pivotal role in the pathophysiological process of uremia and its complications, including cardiovascular disease (CVD) [7]. Superoxide dismutase (SOD) and catalase are the most important enzymatic antioxidant systems in the body. SOD, as the first and most important line of defense against reactive oxygen metabolites (ROM), transforms superoxide ion to H₂O₂ that is a less reactive molecule [8].

Material and methods

In this study, 25 patients with moderate chronic kidney disease and 25 with severe chronic kidney disease patients along with 25 control subjects were taken. Patients with chronic kidney disease (CKD) were chosen from the outpatient clinic of kidney in Mayo Hospital. Blood taken was subjected to

centrifugation in order to separate serum within one hour after collection of blood. The sample was processed and analyzed for the estimation of renal parameters (urea, creatinine and uric acid), lipid peroxidation (LPO), antioxidant enzymes activity (SOD, Catalase and Glutathione). Renal parameters (urea, creatinine and uric acid) was measured by the enzymatic kit method. GSH count was assessed by the method of Tietze [9]. Catalase activity was measured by the method of Aebi *et al.* [10].

Superoxide dismutase (SOD) activity was determined by the method of Kakkar [11]. Malondialdehyde (MDA) in tissue was estimated by the method of Ohkawa [12]. Measurements were made spectrophotometrically. Oxidative stress parameters (catalase, SOD, GSH and MDA) and renal parameters (urea, creatinine and uric acid) in the control group were compared with the same parameters of blood samples of moderate and severe CKD. In statistical analysis, SPSS software was used.

Table I: Descriptive Statistics (Mean \pm SD)**Oxidative Stress Parameters**

Groups	Parameters analyzed			
	Catalase	SOD	MDA	GSH
Control	163.64 \pm 11.56	9.55 \pm 2.21	3.76 \pm 1.25	9.96 \pm 2.47
Moderate CKD	154.13 \pm 1.43	5.73 \pm 1.64	2.33 \pm 0.82	4.32 \pm 1.74
Severe CKD	154.63 \pm 0.81	3.64 \pm 1.39	1.30 \pm 0.84	2.71 \pm 1.17

Table II: Descriptive Statistics (MEAN \pm SD)**Renal Parameters**

Groups	Parameters analyzed		
	Urea	Creatinine	Uric acid
Control	32.75 \pm 4.84	0.91 \pm 0.18	5.03 \pm 0.74
Moderate CKD	88.39 \pm 32.77	2.72 \pm 1.34	7.27 \pm 2.60
Severe CKD	281.88 \pm 102.23	5.67 \pm 1.79	19.38 \pm 7.29

Table III: Multiple Comparison**Oxidative Stress Parameters**

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.
Catalase (CAT)	Control	Moderate	9.50688*	1.98773	0.000
		Severe	9.01225*	2.10456	0.000
	Moderate CKD	Control	-9.50688*	1.98773	0.000
		Severe	-0.49463	1.78534	0.783
	Severe CKD	Control	-9.01225*	2.10456	0.000
		Moderate	0.49463	1.78534	0.783
Superoxide dismutase (SOD)	Control	Moderate	3.81522*	0.61124	0.000
		Severe	5.90882*	0.64716	0.000
	Moderate CKD	Control	-3.81522*	0.61124	0.000
		Severe	2.09361*	0.54900	0.000
	Severe CKD	Control	-5.90882*	0.64716	0.000
		Moderate	-2.09361*	0.54900	0.000
Malondialdehyde (MDA)	Control	Moderate	1.43188*	0.33664	0.000
		Severe	2.46078*	0.35642	0.000
	Moderate CKD	Control	-1.43188*	0.33664	0.000
		Severe	1.02890*	0.30236	0.001
	Severe CKD	Control	-2.46078*	0.35642	0.000
		Moderate	-1.02890*	0.30236	0.001
Glutathione (GSH)	Control	Moderate	5.64058*	0.63643	0.000
		Severe	7.25490*	0.67384	0.000
	Moderate CKD	Control	-5.64058*	0.63643	0.000
		Severe	1.61432*	0.57163	0.007
	Severe CKD	Control	-7.25490*	0.67384	0.000
		Moderate	-1.61432*	0.57163	0.007

TABLE IV: Multiple Comparison

Renal Parameters					
Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.
Urea	Control	Moderate	-55.64130*	22.24042	0.016
		Severe	-249.13235*	23.54764	0.000
	Moderate CKD	Control	55.64130*	22.24042	0.016
		Severe	-193.49105*	19.97584	0.000
	Severe CKD	Control	249.13235*	23.54764	0.000
		Moderate	193.49105*	19.97584	0.000
Creatinine	Control	Moderate	-1.80942*	0.48724	0.001
		Severe	-4.75980*	0.51588	0.000
	Moderate CKD	Control	1.80942*	0.48724	0.001
		Severe	-2.95038*	0.43763	0.000
	Severe CKD	Control	4.75980*	0.51588	0.000
		Moderate	2.95038*	0.43763	0.000
Uric acid	Control	Moderate	-2.18797	1.61500	0.182
		Severe	-14.29667*	1.70993	0.000
	Moderate CKD	Control	2.18797	1.61500	0.182
		Severe	-12.10870*	1.45056	0.000
	Severe CKD	Control	14.29667*	1.70993	0.000
		Moderate	12.10870*	1.45056	0.000

Results and discussion

The mean values of oxidative stress parameters (catalase, SOD, MDA and GSH) in moderate CKD, severe CKD and control group are presented in Table I and the mean values of renal parameters (urea, creatinine and uric acid) in moderate CKD, severe CKD and control group are presented in Table II. Table III shows the comparison among the stress parameters and Table IV shows the comparison among the renal parameters.

In the present study the effects of chronic kidney disease (CKD) on renal catalase and SOD activities as well as renal GSH and MDA levels were evaluated. Data showed that in spite of a decrease in renal GSH, SOD and catalase activity in CKD condition, MDA activity was also decreased. According to descriptive statistics, the mean value of catalase in control, moderate and severe were 163.64 ± 11.56 , 154.13 ± 1.43 and 154.63 ± 0.81 respectively. The observed mean value of SOD in control was 9.55 ± 2.21 , in moderate it was 5.73 ± 1.64 while in severe it was 3.64 ± 1.39 . The mean value of MDA in control, moderate and severe group were 3.76 ± 1.25 , 2.33 ± 0.82 and 1.30 ± 0.84 respectively. GSH in control was observed as 9.96 ± 2.47 , in moderate CKD it was 4.32 ± 1.74 while in severe CKD the observed mean value of GSH was 2.71 ± 1.17 (Table I). Significant increase in both BUN and creatinine showed that CKD resulted in serious renal injury. According to descriptive statistics, the mean value of urea in control, moderate and severe group were 32.75 ± 4.84 , 88.39 ± 32.77 and

281.88 ± 102.23 respectively. The observed mean value of creatinine in control was 0.91 ± 0.18 , in moderate CKD it was 2.72 ± 1.34 while in severe CKD it was 5.67 ± 1.79 . The mean value of uric acid in control, moderate CKD and severe CKD group was 5.03 ± 0.74 , 7.27 ± 2.60 and 19.38 ± 7.29 respectively (Table II). Multiple comparison showed significant difference ($p < 0.05$) in all the parameters (catalase, SOD, MDA and GSH) of oxidative stress assessment as compare to control group and as compare to each other except for catalase levels where insignificant difference ($p > 0.05$) was observed among moderate and severe CKD (Table III). Multiple comparison showed significant ($p < 0.05$) difference in serum creatinine and urea levels in severe CKD and moderate CKD patients as compare to control group. For moderate group insignificant difference in serum uric acid level ($p > 0.05$) was observed as compare to control group while serum uric acid level of severe group showed significant alteration as compare to control group. However significant difference in all the parameters was observed among both the case groups (severe CKD and moderate CKD) (Table IV).

In present study, there was a decrease in renal GSH in chronic kidney disease, as had been shown in other studies [13-15]. This decrease in GSH level could be explained by its consumption in scavenging free radicals and maintaining the redox state of the cell during CKD [14-16]. Decrease in renal catalase activity in CKD in present study is similar to other published data, which remarked decrease in catalase

activity as well as a reduction of its gene expression after renal failure [13]. Significant increase in both BUN and creatinine showed that CKD resulted in serious renal injury. Similar results were obtained in previous studies [17-18]. Decreased effectiveness of the intracellular and plasma enzymatic and non-enzymatic antioxidant protection systems contribute to increased oxidative stress [19-20]. Superoxide dismutase is the first line of the intracellular antioxidant system [21]. The most active antioxidant of non-enzymatic origin is GSH, which is the scavenger for H₂O₂, OH⁻ ions, and chlorinated oxidants [22]. Reduced glutathione is a low molecular thiol (SH) involved as substrate for GSH-Px, which generally prevents protein SH groups from oxidation and cross-linkage [23].

Various authors have found high MDA levels in CKD patients on HD [24]. The end-products of protein and carbohydrate oxidation, malonylhyaldehyde and glutathione hydroperoxidase, in 162 patients with GFRs ranging from 80 to 20 mL/min (1.33 to 0.33 mL/s). They found that levels of end products of protein oxidation were higher compared with controls and correlated inversely with glomerular filtration rate (GFR) [25]. Moreover, CAT and SOD activities were increased, as well as the GSH levels, to compensate for the oxidative stress that results from the hemo-dialysis process [26-27].

Acknowledgements:

The authors are thankful to Ms. Rabail Alam, Lecturer, Institute of Molecular Biology and Biotechnology, The University of Lahore Pakistan for her help in statistical analysis of the data and Dr. Qurban Ali Assistant Professor, Centre of Excellence in Molecular Biology, University of the Punjab Lahore, Pakistan for proof reading of manuscript.

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3/16/2015