academicJournals

Vol. 4(2), pp. 28-33, July, 2013
DOI: 10.5897/JMLD2013-0067
ISSN 2141-2618 ©2013 Academic Journals
http://www.academicjournals.org/JMLD

Journal of Medical Laboratory and Diagnosis

Full Length Research Paper

Role of oxidative stress in aggravating kidney dysfunction in coronary artery disease patients- A laboratory finding

Ritu Sharma* and Mridula Mahajan

Department of Biochemistry, Government Medical College, Amritsar, India.

Accepted 10 June, 2013

Acute cardiac decompensation results in activation of hemodynamic and neurohormonal factors that lead to an acute drop in glomerular filtration rate (GFR) resulting in acute kidney injury. This relationship is referred to as cardiorenal syndrome type 1 (CRS), which usually goes unnoticed. The present study was designed to evaluate the occurrence of kidney dysfunction in coronary artery disease (CAD) patients visiting the clinical biochemistry laboratory. Ninety percent (90%) of CAD patients were observed to have stage 3 chronic kidney disease (CKD) on the basis of their GFR. They had significantly raised ($p < 0.05$) blood urea and serum creatinine levels as compared to those without kidney dysfunction and healthy controls. Renal dysfunction was more pronounced in CAD patients suffering from congestive heart failure along with hypertension. All these patients were advised serum uric acid estimations by the clinician. A close look at serum uric acid levels interestingly showed that levels were significantly low ($p < 0.05$) in CAD patients having kidney dysfunction as compared to those without kidney disease and healthy controls. Uric acid is an important antioxidant molecule in the body. CAD patients with stage 3 CKD had relatively increased oxidative stress as revealed from their low serum superoxide dismutase (SOD) and catalase activity which might lead to quenching of uric acid resulting in its low concentrations. It was proposed that the reduced free radical scavenging capacity of the body may be responsible for inflammatory conditions prevailing in the body in response to the injury to the cell membrane and hence causing organ dysfunction which could be the involvement of kidneys in CAD patients leading to CRS type1. Hence it is very important to check the pro-oxidant-antioxidant balance at the very initial stages.

Key words: Cardio-renal syndrome, creatinine, urea, coronary artery disease, oxidative stress

INTRODUCTION

Heart and kidney share responsibility for maintaining hemodynamic stability through a tight-knit relationship that controls cardiac output, volume status and vascular tone. The reactive oxygen species initiate a cascade of events leading to inflammatory response and hence compromised cellular function. Primary disorders of either heart or kidneys often results in secondary dysfunction or injury to the other. Such interaction represents the patho-physiological basis for a clinical entity called cardiorenal syndrome (CRS). There are evidences of risk and occurrence of kidney dysfunction along with coronary artery disease which at times remains undiagnosed and unattended.

In CRS type 1, acute cardiac decompensation results in activation of hemodynamic and neurohormonal factors that lead to an acute drop in glomerular filtration rate

*Corresponding author. E-mail: rs.gcm1977@gmail.com.

(GFR) and hence the development of acute kidney injury. Approximately 25% of patients with chronic heart failure have been found to have reduced GFR (Hillege et al., 2006). In one of the studies, it has been reported that 21% of patients with heart failure had their serum creatinine concentrations at more than 2 mg/dl and 9% had more than 3 mg/dl (Adams et al., 2005). The reduction in kidney function has significant impact on both morbidity and mortality (Wencker, 2007).

A study by Dammon and colleagues showed that congestive heart failure is associated with increased markers of tubulointerstitial damage such as N-acteyl-beta-D-glucosamine (NAG), kidney injury molecule-1(KIM) and neutrophil gelatinase associated lipocalin (NGAL) (Damman et al., 2007). Acute CRS type1 is more frequent in patients suffering from acute decompensated heart failure (Eren et al., 2012). The cornerstone of CRS therapy is the early identification of worsening kidney function. Various workers recommended the use of certain potential biomarkers such as cystatin-C, brain natriuretic peptide (BNP), IL-18 and fatty acid binding protein (Haase et al., 2009). These parameters might provide significant information of the tubulointerstitial damage in dysfunction in coronary artery disease (CAD) patients. The novelty of new biomarkers of kidney disease cannot be questioned but a critical role of oxidative stress in the initiation and progression of any disease could also not be ignored. Hence, checking the major culprit that is, oxidative stress at initial stages may be more beneficial in controlling the kidney dysfunction in CAD patients and vice versa.

In addition to other antioxidant molecules, the role of uric acid as an antioxidant has come into picture. In humans, it contributes as much as 2/3 of all the free radical scavenging capacity in plasma (Squadrito et al., 2000). It seems that too much lowering of uric acid in the body due to any reason could create prooxidant-antioxidant imbalance and probably contribute in the organ dysfunction. The aim of the present study was to evaluate the occurrence of renal dysfunction in CAD patients (North West Punjabi population) visiting the Clinical Biochemistry Laboratory for their routine investigations. It is pertinent to mention that these patients were confirmed cases of CAD and no diagnosis of their kidney dysfunction was made earlier.

MATERIALS AND METHODS

The present study was conducted in the Clinical Biochemistry Laboratory of Guru Nanak Dev Hospital (attached hospital of Government Medical College) Amritsar, India. A total of hundred (n = 100) CAD patients visiting the clinical biochemistry laboratory were selected. The diagnosis of CAD was done by the clinician on the basis of clinical symptoms, electrocardiography (ECG) changes and treadmill test (TMT) wherever required. The data of 50 normal healthy subjects (from our previous studies) serving as control group was included for reference (Sharma et al., 2006). CAD patients with diagnosed diabetes mellitus, thyroid disease, gout or any acute infection were excluded. All the patients were screened for serum total lipid profile, plasma serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood glucose and serum uric acid levels along with blood urea and serum creatinine to evaluate their kidney functions. Their demographic data was obtained from their case history files. GFR was calculated by modification of diet in renal disease (MDRD) formula and patients were considered for different clinical stage of chronic kidney disease (CKD) as per kidney disease outcomes quality initiative guidelines (NKF-KDOQI, 2000). Written informed consent was obtained from all the patients as per The Ethical Committee of the Institute. Serum super oxide dismutase (SOD) activity was estimated by the method of Marklund and Marklund (1988). Serum catalase activity was assayed by the method of Aebi (1984). Routine biochemical investigations were done with commercially available kits (Alpha-Chem, Harayana, India) on semi-auto-analyzer (Tran-Asia) and were validated against the reference sera. Student's *t*-test was applied to check the significance at level $p < 0.05$.

RESULTS

Table 1 shows the percentage of CAD patients suffering from kidney dysfunction. Out of 100 CAD patients visiting the clinical biochemistry laboratory, 90% were observed to have kidney dysfunction which was revealed from their blood urea and serum creatinine concentrations. In these patients, mean blood urea levels were 88 ± 5.2 mg/dl and those of creatinine were 7 ± 2.0 mg/dl. Both these levels were significantly raised ($p < 0.05$) as compared to controls and CAD patients without kidney dysfunction. The data clearly indicates the increased risk of kidney dysfunction in patients suffering from CAD. GFR of all the CAD patients with and without renal dysfunction was calculated with MDRD formula (ref) in order to evaluate the clinical stage of CKD. Further, CAD patients were screened for various biochemical parameters and their demographic data was obtained from the records (Table 2). Body mass index (BMI) of CAD patients with or without chronic kidney disease was not significantly different from controls. However, GFR of CAD patients with chronic kidney disease was in the range of 30 to 59 ml/min, hence these patients were considered as stage 3 CKD as per Kidney Disease Outcomes Quality Initiative guidelines (2000). Majority of the CAD patients (n = 60) were on cardiac and antihypertensive treatment. Total lipid profile that is, levels of serum total cholesterol, triglycerides, very-low-density lipoprotein (VLDL-C), low-density lipoprotein (LDL-C) and serum high-density lipoprotein (HDL) cholesterol levels of these patients were not significantly different ($p > 0.05$) in comparison to controls (Table 2). Similar results were observed in case of SGOT, SGPT, alkaline phosphatase and fasting blood glucose levels

Table 3 showed antioxidant profile of CAD patients with stage 3 CKD. These patients had significantly low ($p < 0.05$) levels of serum uric acid, SOD and catalase activity in comparison to normal subjects (controls) and CAD patients without kidney dysfunction. This data clearly

Table 1. Percentage of CAD patients having kidney dysfunction.

Subject	Parameter	Blood urea (mean±SD)	Serum creatinine (mean±SD)
Controls (n=50)	No kidney dysfunction	24±3.0	0.5±0.9
CAD patients (n=100)	10% Kidney dysfunction	(-)28±1.2	0.6±0.08
	90% Kidney dysfunction (Stage 3 CKD)	(+)88±5.2*	7±2.0*

Results were expressed as Mean±SD mg/dl; *p < 0.05: significantly increased blood urea and serum creatinine levels in CAD patients with kidney dysfunction (Stage 3 CKD) as compared to those without kidney dysfunction and control group comprising healthy subjects.

indicate the low antioxidant potential in CAD patients with severe kidney dysfunction and increased risk of further complications.

CAD patients with stage 3 CKD were further classified into three groups on the basis of their differential diagnosis to have a better idea of their renal functions as well as their antioxidant status (Table 4). In Group 3 patients (CHF+HTN), blood urea and serum creatinine levels were significantly ($p < 0.05$) elevated as compared to the patients in other two groups, suggesting that group 3 patients suffering from congestive heart failure along with hypertension had more severe kidney dysfunction as compared to the patients in other two groups. On the same lines, serum uric acid levels were also significantly low ($p < 0.05$) in these patients in comparison to the other two groups. However, serum SOD and catalase activities did not show significant difference ($p > 0.05$) among the three patient groups. However, it is pertinent to mention here that overall, all the CAD patients with stage 3 CKD were having significantly low ($p < 0.05$) serum SOD and catalase activity as compared to controls and patients without kidney dysfunction as mentioned in Table 3. It may be noticed that these activities of antioxidant enzymes were compared with that of normal healthy subjects in studies done previously in our own laboratory (Table 3) (Sharma et al., 2006).

DISCUSSION

A strong communication exists between heart and kidneys through a variety of pathways. The mediators of these pathways include the sympathetic nervous system, the rennin-angiotensin-aldosterone axis and arterial natriuretic peptide. In the setting of underlying heart disease or chronic kidney disease, the capacity of each organ to respond to perturbation caused by the other may become compromised. This has led to the characterization of the cardiorenal syndrome (CRS). The present study was an observational approach on the blood samples of CAD patients received in the clinical biochemistry laboratory for analysis. It may be noticed that these patients were confirmed cases of CAD and their their renal status was unknown as no diagnosis of any CKD was mentioned on their outpatient department (OPD)/ward medical cards. All these patients were from various wards and outpatient departments of the Department of Medicine who were under treatment by the physician. It was observed that in all of these diagnosed and treated cases of CAD, lipid profile and the enzymatic estimations were in a normal reference range whereas the disturbances in the routine biochemical investigations such as blood urea and serum creatinine were quite prominent, hence bringing to attention the current developments in literature providing evidences for occurrence of renal failure in these CAD patients.

Approximately 90% of CAD patients were having significantly raised blood urea and serum creatinine levels when compared to the that of healthy controls and it was observed to be more prominent when the values were compared to that of rest 10% patients of coronary artery disease patients who were having normal kidney functions as suggested by their normal levels of blood urea and serum creatinine. These 90% CAD patients were considered as stage 3 CKD on the basis of their estimated GFR calculated with MDRD formula and as per Kidney Disease Outcomes Quality Initiative guidelines (2000). Besides, renal dysfunction was observed to be more severe in patients having congestive heart failure along with hypertension. Heart failure is a common chronic condition affecting 2% of the adult population (Mcmurray et al., 2005). The decompensated heart failure results in reduced effective arterial filling volume (Schrier and Abraham, 1999). The CHF patients in the present study were decompensated and this information was revealed from their Medical case history files.

All the patients were advised serum uric acid estimations by the clinician. A close look at the values of serum uric acid interestingly revealed that the uric acid levels were significantly low in CAD patients having disturbed renal function than those having relatively better renal profile as well as the controls. All these findings are suggestive of the fact that probably oxidative stress is playing a significant role in causing injury to the cellular membrane (reaction to injury hypothesis), hence the inflammatory response in the initial phases of coronary

Table 2. Demographic data and Biochemical profile of CAD patients (with and without kidney dysfunction) and controls

Parameter	Age (years)	Male:female	BMI (kg/m²)	GFR (ml/min)	Blood pressure (mm/Hg)	Treatment	Serum total cholesterol	Serum TG	Serum VLDLC	Serum LDL-C	Serum HDL-C	SGOT	SGPT	ALP	Glucose
Control	42±5	22:28	24±4	80-125	<140/90	-	200±5.0	130±6.0	27±3.0	128±5.0	45±3.0	28±4.2	30±5.0	80±4.8	70±6.0
CAD patients with stage 3 CKD	45±5	32:58	25±5	30-59	>140/90	+	**180**±4.2	124±3.0	24±2.8	115±5.0	41±1.6	27±3.2	28±4.1	73±3.5	80±4.0
CAD patients without CKD	43±6	4:6	26±2*	75-100	>140/90	+	210±5.0*	130±5.2*	28±3.0*	140±4.0*	42±3.0*	30±3.8*	24±5.3*	82±4.0*	84±2.0*

*p > 0.05 Insignificant difference in different parameters among the three groups; CAD patients were on cardiac and antihypertensive treatment.

artery disease. This was supported from the fact that serum uric acid levels, SOD and catalase activities were significantly lower in CAD patients with stage 3 CKD as compared to the normal healthy subjects enrolled in our previous reported studies (Sharma et al., 2006) and patients without kidney dysfunction (Table 3).

The role of oxidative stress leads to reaction to injury hypothesis and has already been reported by number of workers in CAD (Puddu et al., 2012). It was quite evident from the literature that the oxidation of LDL by free radicals initiates a cascade of events leading to increased inflammation and injury to the cardiac membrane (Manzano-Leon et al., 2013). Similar events of inflammation could produce injury to the membrane of the kidneys also. Acute kidney injury may complicate one-third of the admissions and a 22% higher mortality rate through adversely affecting cardiac performance through electrolyte dysequilibration, volume overload, and negative inotropy (Wencker, 2007). Hence the involvement of kidneys in CAD and vice versa cannot be ruled out.

Mahajan et al. (2009) reported an important role of uric acid as free radical scavenger, better than vitamin C in patients suffering from rheumatoid arthritis. Urates possess preventive antioxidant property in addition to the chain breaking antioxidant activity (Waring et al., 2001). Since uric acid concentrations in plasma of humans are much more than that of plasma ascorbic acid, uric acid contributes more to the scavenging action of free radicals than ascorbic acid. In the extracellular environment, uric acid behaves as a powerful antioxidant, particularly it scavenges peroxynitrite radicals and it is the nature of host environment which decides its role as pro-oxidant or antioxidant (Kuzkaya et al., 2005). Oxidative stress causing injury to the glomerular membrane might be responsible for compromised glomerular functions, and decreased glomerular perfusion or decreased cardiac output activates the rennin-angiotensin system, nitric oxide, adenosine and prostaglandin production to prevent dramatic changes in kidney function (Tang and Mullens, 2010).

Renin angitensin system activation results in increased AII which stimulates nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Griendling et al., 1994). The resulting NADPH/NADH suppresses superoxide dismutase and increases reactive oxygen species. In the present study too, a significant decrease in serum SOD and catalase activity was observed in CAD patients irrespective of whether they were having renal dysfunction or not. Compromised antioxidant functions result in the well known cascade of hypoxic ischemic injury, inflammation, apoptosis and cell death (Griendling et al., 1994). Moreover, it has been suggested that there is defective regulation of monocyte apoptosis in patients suffering from CRS type 1 leading to increased inflammation and oxidative stress (Virzi et al., 2012).

The early identification of worsening kidney function is highly essential. Various biomarkers such as NGAL, NAG and KIM-1 have been implicated in the tubulointerstitial damage and have been used to identify acute kidney injury (Haase et al., 2009). Serum cystatin C is a marker of reduced glomerular filtration while urinary Cystatin C is a marker of tubular dysfunction (Herget-Rosenthal et al., 2007). In addition to these novel biomarkers, we suggest that the need of the hour appears to bring a check to the disturbed antioxidant status in CAD patients so that the multiorgan involvement especially that of kidneys can be controlled at the earliest when CAD is diagnosed. Uric acid could act as promising parameter in this respect. The evaluation of the trend of uric acid levels with the course of either CAD or renal disease might provide significant information of the underlying oxidative stress and hence inflammation.

Ninety cases out of hundred represent only a fraction of patient population suffering from CRS type 1 and the number may be many folds more in the actual conditions.

Table 3. Antioxidant profile of CAD patients with stage 3 CKD

Antioxidant parameter	Control	CAD patients with stage 3 CKD	CAD patients without kidney dysfunction
Serum uric acid (mg/dl)	5.8±1.2	3.8±1.0*	5.2±0.8
Serum SOD activity (IU/ml)	6.4±1.3	2.1±0.4*	4.5±1.0
Serum Catalase activity	7.8±2.0	4.0±1.1*	6.2±0.6

*p < 0.05: Significantly low uric acid levels, SOD and catalase activity in CAD patients with stage 3 CKD as compared to controls and patients without kidney dysfunction.

Table 4. Increased oxidative stress and renal dysfunction in patients suffering from coronary artery disease (CAD patients with stage 3 CKD).

Parameter	CAD patients with renal dysfunction		
	Group1 (CAD cases) (n=30) [Range; M±SD]	Group 2 (CAD+HTN) (n=25) [Range; M±SD]	Group3 (CHF+HTN) (n=35) [Range; M±SD]
Serum uric acid (mg/dl)^	3.5-5.5; 4.5±1.0	3-5.0; 4.2±1.2	2.5-4.2; 3.5±0.98*
Serum SOD activity	1.5-3.5; 2.4±0.59	2-3.0; 2.5±0.7	1.8-3.2; 2.4±0.82**
Serum catalase activity	2-5.6; 4.6±1.2	2.5-5.7; 4.1±1.5	2.5-4.8; 3.5±1.6**
Blood urea (mg/dl)^	55-88; 75±5.1	56-98; 82±3.4	67-120; 109±5.2*
Serum creatinine (mg/dl)^	1.3-4.0; 2.8±1.3	1.6-6.8; 5.0±1.8	3.2-10.5; 8±2.0*

*p<0.05 significantly raised levels of serum uric acid, blood urea and serum creatinine in Group 3 patients as compared to other two groups whereas serum SOD and catalase activities showed insignificant difference in this respect (**p>0.05). HTN: Hypertensin; CHF: congestive heart failure.

Secondly, in this observational study only 10% of CAD cases were observed to have normal renal functions in comparison to 90% who were having disturbed kidney function, hence less number of cases in the former could be the limitation of the study, however, indirectly this ratio suggest the increased occurrence of renal dysfunction in CAD. The observation of normal cardiac profile as well as other enzyme activities could be the result of the treatment given to these patients. An important point to discuss over here is that blood urea and serum creatinine levels were not normal, rather they were significantly elevated, this clearly indicates that CRS at times remains undiagnosed and unattended to (quite evident from the present study) which could add complexities in the disease management.

Since it was an observational study and not a comparative one, hence the robustness of blood urea, serum creatinine and uric acid over other new biomarkers cannot be questioned. The results obtained from the present study is only a small representation of the population actually suffering from CRS, hence more and more data is required to evaluate the role of oxidative stress in diagnosis and management of cardio-renal syndrome. A follow-up study aiming at investigating the uric acid levels in a healthy population would be beneficial to have a better idea of its variations during a course of acquired CAD or CKD. Further, no direct marker of inflammation has been evaluated because of the observational nature of the present study but certainly on the basis of these findings, a case-control study would be planned taking into account the novel markers of oxidative stress and inflammation in evaluating CRS.

ACKNOWLEDGEMENTS

Authors are thankful to the patients for their valuable support and cooperation.

REFERENCES

Adams KF, Fonarow JC, Emerman CL (2005). Characteristics and outcomes of patients hospitalized for heart failure in the United States: rationale, design and preliminary observations from the first 100,000 cases in the acute decompensated heart failure National registry (ADHERE). Am. Heart J. 149:209-216.

Aebi H (1984). Catalase *in vitro*. Methods Enzymol. 105: 121-126.

Damman K, Navis G, Smilde TD, Voors AA, van der Bij W, van Veldhuisen DJ, Hillege HL (2007). Decreased cardiac output, venous congestion and the association with renal impairment in patients with cardiac dysfunction. Eur. Heart J. 9: 872-878.

Eren Z, Ozveren O, Buvukoner E, Kaspar E, Degertekin M, Kantarci G (2012). A single control study of acute cardiorenal syndrome: incidence, risk factors and consequences. Cardiorenal Med. 2:168-176.

Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW (1994). Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ. Res. 74:1141-1148.

Haase M, Bellomo R, Devarajan P, Schiattmann P, Haase-Fielitz A (2009). Accuracy of neutrophil gelatinase-associated lipocalin in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am. J. Kidney Dis. 54:1012-1025.

Herget-Rosenthal S, Van Wijk JAE, Brocker-Preuss M, Bokenkamp A

(2007). Increased urinary cystatin C reflects structural and functional renal tubular impairment independent of glomerular filtration rate. Clin. Biochem. 40:13-14.
Hillege HL, Nitsch D, Pfeffer MA, Swedberg K, McMurray JJ, Yusuf S, Granger CB, Michelson EL, Ostergren J, Cornel JH, de Zeeuw D, Pocock S, van Veldhuisen DJ (2006). Renal function as a predictor of outcome in a broad spectrum of patients with heart failure. Circulation 113:671-678.
Kuzkaya N, Weissmann N, Harrison DG, Dikalov S (2005). Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase. Biochem. Pharmacol. 70:343-354.
Mahajan M, Kaur S, Mahajan S, Ravi K (2009). Uric acid a better scavenger of free radicals than vitamin C in Rheumatoid Arthritis. Indian J. Clin. Biochem. 24:205-207.
Manzano-León N, Mas-Oliva J, Sevilla-Tapia L, Morales-Bárcenas R, Serrano J, O Neill MS, García-Cuellar CM, Quintana R, Vázquez-López I, Osornio-Vargas AR (2013). Particulate matter promotes in vitro receptor recognizable low density lipoprotein oxidation and dysfunction of lipid receptors. J Biochem. Mol. Toxicol. 27(1):69-76.
McMurray JJV, Pfeffer MA (2005). Heart Failure. Lancet 365:1877-1889.
NKF-KDOQI Clinical Practice Guidelines for Nutrition in Chronic Renal Failure (2000). Am. J. Kidney Dis. 35:S1-S140.
Puddu P, Puddu M, Cravero E, Vizioli L, Muscari A (2012). The relationship among hyperuricemia, endothelial dysfunction, and cardiovascular diseases: molecular mechanisms and clinical implications. J. Cardiol. 59:235-242.
Schrier RW, Abraham WT (1999). Hormones and hemodynamics in heart failure. N Engl. J. Med. 341:577-585.
Sharma R, Singh B, Mahajan M (2006). HDL associated paraoxonase1 activity in relation to oxidative stress in CAD patients. Curr. Cardiol. Rev. 2:125-129.
Squadrito GL, Cueto R, Splenser AE, Valavanidis A, Zhang H, Uppu RM, Pryor WA (2000). Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. Arch. Biochem. Biophys. 376:333-337.
Tang WHW, Mullens W (2010). Cardiorenal syndrome in decompensated heart failure. Heart ; 96: 255-260.
Virzì GM, Torregrossa R, Cruz DN, Chionh CY, de Cal M, Soni SS, Dominici M, Vescovo G, Rosner MH, Ronco C (2012). Cardiorenal syndrome may be immunologically mediated: A pilot evaluation of monocyte apoptosis. Cardiorenal Med. 2:33-42.
Waring WS, Webb DJ, Maxwell SR (2001). Systemic uric acid administration increases serum antioxidant capacity in healthy volunteers. J. Cardiovasc. Pharmacol. 38:365-371.
Wencker D (2007). Acute cardio-renal syndrome: progression from congestive heart failure to congestive kidney failure. Curr. Heart Failure Rep. 43:134-138.