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Research article

SERUM LEVELS OF HIGH SENSITIVITY C REACTIVE PROTEIN AND MALONDIALDEHYDE IN CHRONIC KIDNEY DISEASE

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ABSTRACT

Background: Chronic kidney disease cases are at increased risk for progression to end stage renal disease and accelerated atherosclerosis, with premature cardiovascular morbidity and mortality being the more frequent outcome. **Aim:** The study was taken up to find if there is any association between nontraditional cardiovascular risk markers like high sensitivity C reactive protein (marker of inflammation) and malondialdehyde (marker of lipid peroxidation) with the progression of chronic kidney disease. **Methodology**: The study included 44 pre dialysis chronic kidney disease cases and 44 healthy controls. Serum levels of creatinine, high sensitivity C reactive protein and malondialdehyde were estimated in both groups. The mean estimated glomerular filtration rate(eGFR) in chronic kidney disease patients was calculated by the MDRD formula. **Results**: The mean eGFR in cases was found to be 23.65± 14.99 ml/min by MDRD formula. The serum hsCRP and malondialdehyde levels in cases was 11.8± 7.24 mg/L and 3.02 ±1.24 nmol/ml respectively. **Conclusion**: There was a significant negative correlation ($p<0.001$) between high sensitivity C-reactive protein and malondialdehyde with eGFR. A highly significant positive correlation was found between serum hsCRP and malondialdehyde ($p<0.001$) in chronic kidney disease underlining the synergism between oxidative stress and inflammation, perpetuating to further deterioration of renal function and enhancing the predisposition to cardiovascular risk with the progression of chronic kidney disease.

Keywords: Chronic kidney disease, Estimated glomerular filtration rate, High sensitivity C reactive protein, Inflammation, Malondialdehyde, Oxidative stress.

INTRODUCTION

Chronic kidney disease has gained attention as a public health problem worldwide with the increase in incidence and prevalence of the disorder. The Kidney Diseases Outcomes Quality Initiative (K/DOQI) defines chronic kidney disease (CKD) as kidney damage or glomerular filtration rate (GFR) less than 60 ml/min/1.73 m^2 for a period of three months or more, irrespective of cause [1] . The glomerular filtration rate (GFR) is the amount of plasma that is filtered by the glomeruli per unit time and is a reliable measure of the functional capacity of the kidneys. Based on the GFR, CKD is divided into five stages with stage 5 being end stage renal disease having a GFR of <15ml/min. Chronic kidney disease cases with end stage renal disease ultimately undergo renal replacement therapy either in the form of dialysis or renal transplantation. The measurement of GFR is very useful in monitoring the progression of CKD, targeting treatment and predicting renal replacement therapy.

However, cardiovascular morbidity and mortality due to accelerated atherosclerosis is encountered more frequently in CKD patients. CKD is considered an independent risk factor for the development of cardiovascular disease [2]. There is increase in cardiovascular risk whilst there is decline in

glomerular filtration rate below 70 ml/min[3, 4]. The traditional risk factor for development of atherosclerosis includes increase in age, predilection to male gender, hypertension, smoking, diabetes mellitus, dyslipidemia and others[5]. However, these conventional factors are unable to entirely explain the mechanism for the increased risk for atherosclerosis in the CKD population. In some of the CKD cases there is increase in serum triglyceride with associated decrease in serum high density lipoprotein and with no alteration in other fractions of lipoproteins[6].

The uremic milieu of CKD patients contains high amounts of proinflammatory proteins and cytokines like C reactive protein, interleukin 6 and others[7]. Atherosclerosis too is an inflammatory condition of the arteries and C reactive protein (CRP) which is produced chiefly in the hepatocytes under the influence of interleukin 6 (IL-6) and IL-1 is an important inflammatory mediator. Among the various mechanisms responsible for increase in oxidative stress in uremia, activation of reduced nicotinamide adenine dinucleotide oxidase which can be stimulated by angiotensin II is a very important one[8].

There are studies to demonstrate the role of increased levels of CRP and reactive oxygen species in end stage renal disease and patients undergoing dialysis [9, 10]. However, the role of inflammatory protein CRP and oxidative stress markers like malondialdehyde in the progression of renal dysfunction and accelerated atherosclerosis in predialytic patients is not very clear. The study was intended to determine the levels of high sensitivity C reactive protein (hsCRP) as a marker of inflammation and malondialdehyde (MDA), a lipid peroxidation product as a marker of oxidative stress in predialytic renal disease patients and to decipher if there is any association between serum hsCRP and MDA levels with the progression of kidney disease.

MATERIALS AND METHODS

Study design: Cross sectional , case control study

Ethical approval: The study was approved by Institutional Ethics review board; an informed consent was taken from the patients before the collection of blood sample.

Sample size: The study population included 44 healthy control subjects and 44 pre dialytic nephropathy cases who attended the outpatient clinic of the Department of Nephrology of our college.

Inclusion criteria: Clinically diagnosed chronic kidney disease patients with serum creatinine level greater than 1.5mg/dL were chosen. The estimated GFR (eGFR) was calculated based on MDRD formula [11] [186 × (S.creatinine mg/dl)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female)] mL/min/1.73 m^2]

Patients were grouped into:

Stage III CKD – eGFR : 30-59 ml/min

Stage IV CKD – eGFR : 15-29 ml/min

Stage V CKD – eGFR : <15ml/min

Exclusion criteria: Patients with active infection or chronic ongoing inflammation, history of cardiovascular disease, autoimmune disorders, renal transplant and chronic kidney disease on dialysis, drug supplementation such as steroids, immuno suppressants, non steroidal anti - inflammatory drugs, oral contraceptives, hormone replacement therapy, statins, niacin and fibrates were excluded from the study.

Grouping: The participants were divided into two groups:

Group1: Control (Healthy volunteers who visited the hospital for routine health check up and were willing to be part of the study were taken as controls. The control subjects had the same exclusion criteria as CKD cases.)

Group 2: Chronic kidney disease(CKD) cases.

Methodology

5 ml of venous blood sample was collected under aseptic conditions from the study groups in BD vacutainer. The sample was allowed to clot. The serum was separated from the sample at the earliest after centrifugation and used for the estimation of serum creatinine, high sensitivity C - reactive protein and malondialdehyde. Serum creatinine was estimated on Roche/Hitachi COBAS c-501 autoanalyzer by Buffered Kinetic Jaffe reaction without deproteinization (ID-MS traceable method). hsCRP in serum was measured by a turbidimetric immunoassay. The assay is based on a latex-enhanced turbidimetric immunoassay method. The agglutination that occurs between CRP in a sample and anti-CRP antibody which has been sensitized to latex particles, is detected as an absorbance change (570 nm), with the magnitude of the change being proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration. MDA was measured by the Thiobarbituric acid reactive

substances (TBARS) method as described by Wilbur et al [12].

Statistical analysis: The results were expressed as mean ± SD. Significance was assessed at 5% level of significance. Student "t" test (two tailed, independent) and analysis of variance (ANOVA) was used to find the significance of study parameters. Pearson correlation was used to study the relation between the various parameters. Statistical analysis was performed using SPSS 15.0 software.

RESULTS

The study population involved 44 predialytic CKD patients whose average age was 55 ± 13 yrs. The serum creatinine level in controls were within physiological range and in CKD cases the mean serum creatinine level was 4.07± 2.78 mg/dl .The eGFR ranged from 4.48 ml/min to 60.61ml/min in CKD cases with an average of 23.65±14.99 ml/min. In controls the eGFR was found to be 152.33± 41.51ml/min (Table 1). The serum hsCRP levels in cases was 11.8 ±7.24 mg/L with values ranging from 1.9 mg/L to 26.5 mg/L. The hsCRP level was found to be fivefold higher in cases as compared to controls (p<0.001) (Table 1). The serum malondialdehyde level was significantly higher in chronic kidney disease cases (Table 1). The serum levels of inflammatory marker, hsCRP and oxidative stress marker, MDA was five times higher in CKD cases as compared to healthy controls.

The result was analysed by comparing eGFR, Serum hsCRP and MDA in stage III, IV, V CKD (Table 2). There is a gradual increase in serum creatinine from stage 3 to stage 5 CKD. There is doubling of serum hsCRP in stage 4 as compared to stage 3 and three fold increases in stage 5 as compared to stage 3. The hsCRP in stage V is 18.61 ± 3.86 mg/L as compared to stage IV were it is 11.21 ±6.23 mg/L. There is significant rise in hsCRP with the progression of CKD. There is also a gradual rise inS. MDA with the progression of the disease (Table 2).

There was a significant correlation between S. creatinine and eGFR (Table 3). A highly significant negative correlation was also found between S. hsCRP and S. MDA with eGFR (Figure 1, 2). With the deterioration of kidney function, there is increase in serum creatinine, hsCRP and MDA level.

There was a highly significant positive correlation of serum MDA with hsCRP (r=0.642, p<0.001),(Figure 3). With the progression of CKD, the rise in serum MDA, a marker of oxidative stress indicates intensification of inflammation.

Table 1: Comparison of eGFR and biochemical parameters in controls and CKD cases

Biochemical parameters	Controls (n=44) Mean ± SD	Cases(n=44) Mean ± SD	p value
S. Creatinine (mg/dL)	0.59±0.10	4.07±2.78	< 0.001**
eGFR (ml/min)	152.33± 41.51	23.65± 14.99	< 0.001**
S. hsCRP (mg/L)	2.43±0.74	11.8± 7.24	< 0.001**
S. MDA (nmol/mL)	0.55±0.24	3.02 ±1.24	< 0.001**

Table 2: Comparison of biochemical parameters in different stages of CKD.

Parameters	Stage III CKD(n=16)	Stage IV CKD(n=12)	Stage VCKD (n=16)	p value
eGFR (ml/min)	40.72± 8.7	20.46±3.78	8.97 ± 3.11	-
S. Creatinine (mg/dL)	1.8 ± 3.01	3.1± 0.63	7.08 ±2.41	<0.001**
S. hsCRP (mg/L)	5.44± 3.77	11.21±6.23	18.61 ± 3.86	<0.001**
S. MDA (nmol/mL)	2.39± 1.34	2.92 ± 1.28	3.71 ± 0.7	<0.01

Table 3: Pearson correlation of S. creatinine, hsCRP and MDA with eGFR.

Pair	Cases	
	r value	p value
S. Creatinine v/s eGFR	-0.797	< 0.001**
S. hsCRP v/s eGFR	-0.742	< 0.001**
S. MDA v/s eGFR	-0.389	< 0.01

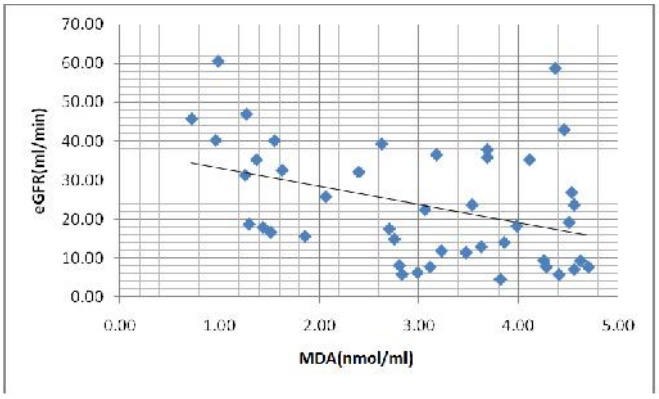

Fig 1: Scatter plot depicting Pearson correlation between MDA (nmol/mL) and eGFR(ml/min)

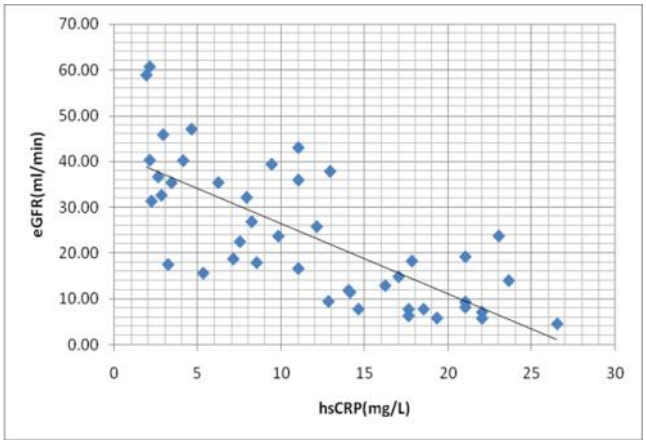

Fig 2: Scatter plot depicting Pearson correlation between hsCRP(mg/L) and eGFR (ml/min)

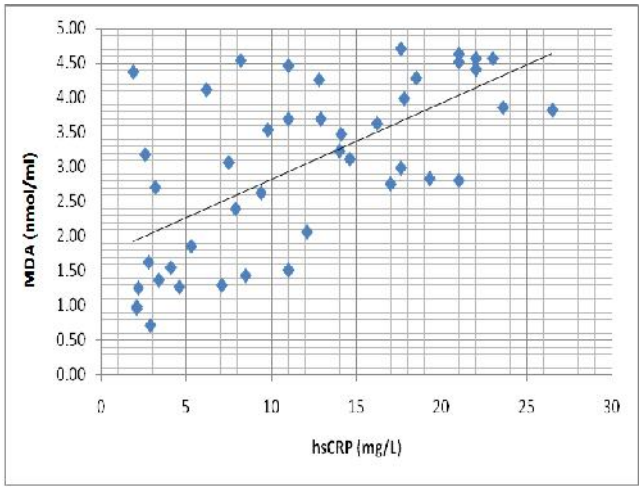

Fig3: Scatter plot depicting Pearson correlation between hsCRP (mg/L) and MDA (nmol/mL)

DISCUSSION

With the rise in both incidence and prevalence of diabetes mellitus in India and the growing inclination towards earlier diagnosis of chronic kidney disease, there is an increase in the number of predialytic CKD patients [13]. CKD patients exhibit an augmented risk for the development of cardiovascular morbidity and mortality which cannot be entirely substantiated by the traditional Framingham risk factors such as age, gender, hypertension, diabetes and hypercholesterolemia. Furthermore, the lipid profile in CKD patients is not very significant as some cases show hypertriglyceridemia which has no definite relation with the various stages of CKD and could be misleading. The uremia related risks include proteinuria, increased renin–angiotensin system activity, chronic volume overload, altered calcium and phosphorus metabolism, inflammation, infection, anemia, malnutrition, oxidative stress, elevated levels of homocysteine, uremic toxins and thrombogenic factors[14].

Oxidative stress and inflammation have received increased importance as nonconventional risk factors of cardiovascular morbidity and mortality in CKD [15]. Oxidative stress is defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant compounds and insufficient antioxidant defense mechanisms. Evaluation of oxidative stress is difficult because free radicals have very short half-lives. However there are more stable marker molecules that have longer half-lives, ranging from hours to weeks, which can be used to assess oxidative stress. Malondialdehyde, a water soluble, three carbon, low molecular weight reactive aldehyde is one such molecule which is a product of the lipid peroxidation and has been studied as an indicator of oxidative stress. Serum MDA levels in CKD cases was found to be significantly higher as compared to healthy controls ($p<0.001$) (Table1). This finding is in agreement with studies by Oberg et al [15], who also reported increase in oxidative stress in CKD patients. Increased generation of MDA as well as its decreased renal clearance due to compromised renal function leads to increased concentration of lipid peroxidation products in circulation in renal failure patients.

The mechanisms of oxidative stress in uremia involves activation of reduced nicotinamide adenine dinucleotide (NAD(P)H) oxidase, xanthine oxidase, myeloperoxidase (MPO), mitochondrial oxidases and uncoupling of endothelial nitric oxide synthase (eNOS) [16]. Furthermore, in CKD there is activation of the renin angiotensin aldosterone axis which leads to the stimulation of NADPH oxidase by angiotensin II [17]. NADPH oxidase is the most important source of reactive oxygen species in the systemic as well as renal vasculature. Angiotensin II activates NADH/NADPH oxidase and protein kinase C activity in vascular cells thereby increasing superoxide ion production and decline in nitric oxide (NO) availability which can lead to endothelial dysfunction in CKD. The reactive oxygen species along with inflammation can cause increase in cardiovascular risk and further deterioration of renal function. The production of reactive oxygen species (ROS) which can occur at constitutive levels in nonphagocytic cells (e.g., glomerular cells and tubular epithelial cells) for

physiological purposes gets deranged and can lead to loss of redox homeostasis and oxidative stress which can contribute to proinflammatory and profibrotic pathways in the kidney. Peroxidation of lipids brings about changes in the molecular structure of the lipids and these changes becomes more marked when the damaged lipids are the constituents of the biological membrane disrupting the cohesive lipid layer arrangement and structural organization. The lipid peroxides in general, enhances prostaglandin synthesis which is another source of free radical and associated decrease in NO production are well known risk factor for atherosclerotic complication.

Formation of ROS is evident in many areas of the kidney, predominantly in the renal cortices, sparing the medulla which is susceptible to hypoxia and less ROS production under physiologic conditions. The substantial generation of ROS is all the more damaging because CKD patients have a weaker antioxidant system which also can be attributed to intake of diet low in antioxidant nutrients. The disturbances in cellular oxidant and pro-oxidant status can cause detrimental effect by altering cellular signaling process and perpetuating renal cell apoptosis and senescence [16]. Due to increased production of reactive oxidants there is predisposition to enhancement in the peroxidation of lipids and lipoproteins. In the study, a graded increase in serum MDA levels are observed with the progression of renal failure. A significant negative correlation between serum MDA levels and eGFR is found in the CKD cases ($p<0.001$) (table 3). Serum malondialdehyde (MDA) a three carbon compound reflects both autoxidation and oxygen mediated peroxidation of poly unsaturated fatty acids in particular. It reflects the oxidative status of the biological system. MDA causes damage to low density lipoproteins (LDL) which in turn can be taken up by macrophages via scavenger receptors and form foam cells. Due to increased production of ROS and increased oxidative stress, lipid peroxidation products are found to be elevated in chronic kidney disease cases. Oxidative stress promotes vascular smooth muscle cell proliferation, hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen. Increased oxidative stress, can initiate further damage to the endothelium thereby causing impairment of endothelium-dependent vascular relaxation and increases vascular contractile activity.

The augmented oxidative stress in CKD leads to a net deficiency of NO. Nitric oxide production and/or bioavailability in the vascular endothelial cells involves normal functioning of endothelial nitric-oxide synthase (eNOS), and optimal concentrations of the substrate L-arginine and the cofactor 5,6,7,8-tetrahydrobiopterin (BH_4). The physiological actions of NO include the regulation of vascular tone and blood pressure, prevention of platelet aggregation and inhibition of vascular smooth muscle proliferation. NO can inhibit the activation of xanthine oxidase and NADPH oxidase. Under pathological conditions such as CKD there is uncoupling of endothelial NO synthase enzyme wherein, electrons are transferred to molecular oxygen instead of L-arginine to produce superoxide rather than NO and also inactivation of nitric oxide that is already available by the increased oxygen free radicals [18]. These oxygen free radicals additionally work on LDL cholesterol molecules leading to their oxidation. Oxidized LDL attack the arterial intima and triggers an inflammatory response in the vessel wall.

The causes for inflammation in CKD are multifactorial. Several markers are studied as inflammatory markers in CKD. These markers can be used to predict future risk of CVD in CKD patients. In the study C reactive protein level, a marker of inflammation in stage III, IV and V of CKD and healthy controls was estimated. hsCRP is an acute phase reactant, produced chiefly in the hepatocytes and its synthesis is transcriptionally driven by interleukin 6 with synergistic enhancement by interleukin 1. CRP is released in response to inflammation produced by numerous external and/or internal stimuli and increases dramatically after severe trauma, bacterial infection, inflammation, surgery or neoplastic proliferation. It is found to bind to the fcγ 1 and 2 receptors on the surface of phagocytic cells and helps in the clearance of the apoptotic and necrotic cells behaving as an opsonin. In an apparently normal individual with no other signs of infection, inflammation or trauma, the basal levels of CRP are detected by a more sensitive assay, as high sensitivity CRP (hsCRP). Serum hsCRP is a well studied marker in inflammation and has advantage for the detection and predictor of inflammation. The mean hsCRP level in CKD cases obtained in the study was 11.8 $\pm$7.24 mg/L which is comparable to earlier studies by G Abraham et al [19].

The hsCRP levels have increased by almost up to five times in nephropathy patients as compared to controls, but did not show any significant correlation with age of the patients studied. Underlying etiology of CKD, such as diabetes or hypertension is by itself a major contributory factor to the existing inflammation. Payson et.al [15] have reported on the renal related causes of inflammation in CKD such as retention of uremic toxins, sympathetic over activity and fluid overload. hsCRP has been well studied as a biomarker indicating increased cardiovascular risk, wherein levels >3mg/L indicate high risk and levels <1mg/L indicate low cardiovascular risk [20]. Raised levels of serum hsCRP between 1-3 mg/L is an indicator of chronic low level inflammation probably arising out of the endothelial dysfunction , predisposing to the pathogenesis of atherosclerosis. High concentration of the protein CRP and its mRNA has been noted in the plaque, as compared to its concentration in the plasma, which could well contribute to the proinflammatory and proatherogenic effects of the protein. Devaraj et al [21] have outlined the active role of CRP in the pathogenesis of atherosclerosis wherein they have demonstrated that CRP can also be synthesized by macrophages, smooth muscle cells and endothelial cells, by noting the presence of both protein as well as its mRNA in the atherosclerotic plaque. CRP can no longer be considered an innocent bystander or biomarker of cardiovascular risk, but is an actively proatherogenic molecule which induces cell adhesion molecules, plasminogen activator inhibitor-1, complement activation and attenuates nitric oxide production. This leads to a state of endothelial dysfunction which is a well known precursor of cardiovascular morbidity [21]. Inflammation, oxidative stress and endothelial dysfunction represent a key triad for the development and progression of atherosclerosis.

The study showed a significant graded increase ($p<0.001$) in the levels of hsCRP in stages III, IV and V CKD. hsCRP levels have more than doubled and tripled in stage IV and V CKD as compared to stage III underlining the highly significant correlation ($p<0.001$) between hsCRP and eGFR. hsCRP can also predispose to inflammation through binding with lipoprotein and activation of the complement system [22]. The rising inflammation may lead to deteriorating renal function which in turn could lead to further increase in inflammation setting up a vicious cycle. hsCRP has been reported to induce adhesion molecule expression in human endothelial cells favoring the involvement of hsCRP in the atherosclerotic process.

The present study shows a significant positive correlation between oxidative stress marker MDA and hsCRP, a marker of inflammation in CKD. Nguyen et al [23], have also found similar results. Nuclear factor kappa B is a redox sensitive transcription factor that leads to induction of genes of various proinflammatory cytokines and adhesion molecules, links oxidative stress with inflammation. Inflammation and ROS together lead to increased propensity for atherogenesis in CKD as reviewed by Cachofeiro et al [24]. hsCRP has been shown both as marker and mediator of atherosclerosis. Patients with CKD have increased mortality which cannot be explained by traditional risk factors like diabetes mellitus, hypertension and others but by nontraditional factors like inflammation, malnutrition and predisposition to infection which can culminate in increased cardiovascular risk in these cases. hsCRP can also be considered as a potent independent predictor of cardiovascular mortality as well as malnutrition. Inflammation per se can contribute to increased cardiovascular risk but there can be increased predisposition to cardiovascular complication in CKD cases due to the inflammatory response.

CONCLUSION

Inflammation and oxidative stress in chronic kidney disease sets in much before dialysis, and furthermore the hsCRP and MDA levels rise significantly with the fall in eGFR. In CKD patients' inflammation and production of reactive oxygen species are linked in a vicious cycle which in turn can cause damage to both the glomerular filtration membrane as well as vascular endothelium, worsening the progressive loss of nephrons as well as triggering atherosclerosis in this population. Better insight into the role of the nontraditional cardiovascular risk factors in various stages of CKD is warranted. Levels of these markers can be used not only for risk stratification but also for management wherein levels can be monitored pre and post therapeutic interventions. Prospective studies involving serial measurements of these biomarkers and cardiovascular outcomes in chronic kidney disease would be more valuable in better patient care.

Conflict of Interest: Nil

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