Study of Levels of Serum Malondialdehyde and Serum High Sensitivity C-Reactive Protein in Chronic Renal Failure Patients - A Hospital-Based Study

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ABSTRACT

BACKGROUND

Kidneys are vital organs for excretory and many other biochemical functions in the human body. Most chronic diseases end up damaging the kidneys, acute to chronic, based on the cause and duration. Chronic kidney disease is a sequence of damages to the renal cells and parenchyma leading to progressive deterioration of kidney function, which eventually develops into terminal stage of chronic kidney failure. Chronic renal failure leads to a pro-oxidant state, which leads to damage to the renal cells and parenchyma and the amount of intracellular oxidative stress or extracellular oxidative stress has a relation to the severity of renal failure either directly or indirectly. The study aimed to find the correlation between high sensitivity c-reactive protein (hs-CRP) to lipid peroxidation product, malondialdehyde (MDA).

METHODS

This prospective study was designed and conducted from January 2018 to December 2019 in the Department of Biochemistry, Government Medical College, Ananthapuramu. The study comprised a total of 70 subjects in the age group of 35 - 65 years. The subjects of the approved study plan were divided into two groups; 35 subjects were healthy controls (group-1), and 35 subjects were chronic renal failure (CRF) patients. A blood sample was collected in Government General Hospital, Anantapuramu.

RESULTS

The sample was analysed for estimation of blood urea, plasma glucose, serum creatinine, Malondialdehyde (MDA) and C-reactive protein (CRP). The mean value of blood urea, serum creatinine, serum hs-CRP, serum MDA was higher in CRF (group-2) patients when compared to healthy controls (group-1) ($p < 0.0001$). We observed a positive correlation between serum MDA and serum creatinine ($r = 0.46832$), hs-CRP ($r = 0.0234$).

CONCLUSIONS

In CRF, oxidative stress is obviously evident, but the inflammation induced oxidative stress which can be corrected if detected early will reduce oxidative damage. Our study shows that there is an elevation in hs-CRP and MDA which confirms the presence of oxidative damage, inflammation and probably inflammation induced oxidative damage.

KEYWORDS

CRF, Oxidative Stress, MDA, Serum Creatinine, hs-CRP

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BACKGROUND

Kidneys are one of the essential organs in the excretory and metabolic systems with varied biochemical activities. Most of the acute or chronic diseases have a direct impact on the functioning of nephrons, which may lead to morbidity or early mortality. Based on the causative factor, the kidney disease is broadly classified into acute or chronic illness. Acute and chronic are sub-classified into other types based on the type of causative factor and assessment of renal functions. Chronic kidney disease is due to progressive deterioration of kidney function, which leads to a terminal stage of renal failure.[1] The worldwide prevalence of CKD (Chronic Kidney Disease) is estimated to be 11 % - 13 %, with a relative risk of mortality in patients with CKD compared to those without CKD, which is between 0.94 % to 5 %.[2] The major risk factors of CKD are diabetes mellitus, hypertension, glomerular nephritis, autoimmune diseases, kidney stones, urinary tract infection[3] and toxic effects of drugs like nonsteroidal anti-inflammatory drugs, vancomycin, cisplatin, sulfadiazine, polymyxins.[4] CRF is associated with increased inflammation and oxidative stress, which play an essential role in developing the cardiovascular disorder, and it represents the leading cause of death in CRF patients. C-reactive protein (CRP) is an acute-phase reactant is synthesized in the Liver. This synthesis is in response to cytokine activation.[5] High Sensitive C-Reactive Protein (hs-CRP) is used as one of the markers for the risk of atherosclerosis. Serum level less than 1 mg / L is considered as low risk. Serum levels of 1 mg / L - 3 mg / L are a borderline risk, and levels of more than 3 mg / L have a high risk of occurrence of MI. If the hs-CRP value is more than 10 mg / L, it indicates a significant acute-phase reaction.[6]

Malondialdehyde (MDA), a three-carbon compound and is a low molecular weight aldehyde produced as a result of free radical attack on Poly-Unsaturated Fatty Acids (PUFA).[7] The estimation of serum MDA is used to assess the level of oxidative stress and free radical damage to the body.[8,9]

This study aimed to find the correlation between serum hs-CRP and lipid peroxidation product MDA.

METHODS

The Institutional Ethical Committee cleared this prospective study design, objective, materials and methods. Study was conducted from January 2018 to December 2019. Consent was taken from the subjects after due education, and the sample was collected after explaining the purpose of the study. The study comprised a total of 70 subjects in the age group of 35 - 65 years. The sample size was selected based on feasibility, availability of cases and the incidence and prevalence of the disease in the region. The subjects were divided into two groups, group-1 consisted of 35 healthy subjects without any history of disease and normal biochemical parameters as controls, and 35 subjects of chronic renal failure diagnosed patients were included in the group-2 as cases. Cases and controls were matched with age and sex.

Exclusion Criteria

Cases suffering from acute renal failure, cases already on dialysis, renal transplant, patients suffering from autoimmune disorders and those who are using Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), immune suppressants, statins, fibrates, niacin, steroids and oral contraceptives, pregnant women were completely excluded from this study. Other diseases implicated as oxidant and inflammatory other than renal origin was completely excluded.

Blood samples were collected at the central lab in Government General Hospital, Ananthapuramu, Andhra Pradesh. 5 ml of peripheral venous blood samples were collected in the early morning after an overnight fast, from the basilica or cephalic veins in cubital fossa, using a syringe with a needle and transferred to a plain vacutainer tube. After collection, the blood sample was centrifuged. Plasma was used for the estimation of plasma glucose by Glucose Oxidase-Peroxidase Method (GOD-POD),[10] Blood urea by Di-Acetyl Monoxime method (DAM method),[11] serum creatinine by Jaffe's method,[12] serum malondialdehyde by Thio-Barbituric Acid method (TBA),[13] serum high sensitivity c-reactive protein was estimated by immunoassay method.[14]

Statistical Analysis

In data analysis, a comparison of parameters between the two groups was made by using an unpaired t-test. The data was entered and compiled in an excel sheet. The data was analyzed and consolidated as mean and Standard Deviation (SD). To analyze the statistical significance, the student t-test was performed utilizing Graph Pad software. The test of the probability of less than 0.05 ($p < 0.05$) was significant.

RESULTS

In our present study, the age of the subjects varied from 35 - 65 years. The mean age in controls was 52.6 ± 6.8 and 53.77 ± 7.7. Among the 35 CRF patients, 22 were males, 13 were females, and 26 patients had diabetes. The subject's characteristics of age and sex are shown in Table 1.

Parameter	Controls (Group-1)	Chronic Renal Failure Patients (Group-2)
Age (years) Mean ± SD	52.6 ± 6.8	53.7 ± 7.7
Sex (Males %) (Female %)	68.5 % 31.4 %	62.8 % 46.4 %
No of diabetics	0	26 (74.2 %)
No of smokers	0	11 (31.4 %)

Table 1. Baseline Characteristics of Subjects Studied

The mean value of serum creatinine, blood urea, serum malondialdehyde, serum hs-CRP (Group-2) is significantly elevated when compared to healthy controls (Group-1), as shown in Table 2. Serum MDA obtained, showed a significant positive correlation with serum creatinine ($r = 0.4683$), and hs C-reactive protein (CRP) shows a positive correlation with serum creatinine, as shown in the Table 3.

Biochemical Parameters	Group 1	Group 2	t Value	p Value
Fasting plasma glucose (mg / dl)	92 ± 7.2	141.5 ± 16.47	16.29	< 0.0001
Post prandial plasma glucose (mg / dl)	124.5 ± 9.9	195.8 ± 50.07	8.264	< 0.0001
Blood urea (mg / dl)	27.9 ± 3.1	87.54 ± 15.9	21.76	< 0.0001
Serum\creatinine (mg / dl)	0.91 ± 0.18	6.36 ± 0.54	62.6	< 0.0001
Serum MDA (µmol / L)	1.29 ± 0.2	4.26 ± 1.04	25.75	< 0.0001
hs-CRP (mg / L)	0.89 ± 0.28	13.6 ± 2.64	28.32	< 0.0001

Table 2. Analytes in Group 1 & Group 2, t-Value & P Value

	r Value	t Value	p Value
hs CRP vs. S. creatinine	0.3010	15.895	< 0.0001
MDA vs. Creatinine	0.4683	11.112	< 0.0001

Table 3. Correlation of MDA, hs-CRP, Creatinine with t-Value & P-Value

DISCUSSION

Chronic Kidney disease results in progressive reduction in renal function. CKD is becoming a significant health problem and is rapidly assuming importance of epidemic proportions globally. India has the highest number of diabetics globally, with a prevalence of 3.8 % in rural and 11.8 % in urban. It is associated with unfavourable outcomes in all stages of CKD.[15] In this study, the mean value of serum blood glucose levels (fasting and postprandial) was high when compared to healthy controls ($p < 0.0001$). Our study findings are consistent with previous studies by Neelofer KM et al,[15] Pandya D et al.[15] Diabetes mellitus is established as a metabolic disease that causes multi organ failure, and renal failure increases or decreases the requirement of insulin in diabetic patients. The accumulation of toxins due to uraemia leads to increased levels of parathyroid hormone in chronic renal failure patients, increase in insulin resistance in cells and tissues, usually of skeletal muscle tissues. This is attributed to the damage in the process after insulin binding to its receptors, which alters glucose metabolism and glycogen production.

Secretion of insulin is also seen to be reduced in patients of CRF, and appears to be due to metabolic acidosis and decreased levels of vitamin D. Oxidative stress also plays a pivotal role in the progress of the development of diabetic kidney disease. The average number of patients with diabetic kidney disease is increasing on regular basis worldwide.

An increase in the level of Reactive Oxygen Species (ROS), usually induces oxidative stress and has been considered the primary cause of renal failure. Added to diabetes, renal failure per se also increases oxidative stress. Several macromolecules are implicated, in the generation of reactive oxygen species, including specific defects occurring in the polyol pathway, glycolysis, advanced glycation, xanthine oxidase, reduced nicotinamide adenine dinucleotide phosphate oxidase or uncoupling of nitric oxide synthase, which are the contributors of diabetic kidney disease.[15] High glucose levels mostly lead to damage to millions of nephrons resulting in the overpressure on nephrons and making the kidneys less competent to maintain fluid and electrolyte homeostasis.[16]

In our study, the mean value of creatinine was higher when compared to controls ($p < 0.0001$). The findings were similar to previous studies by SN Sridhar A Veta,[2] Pandya D et al.[16] The healthy range of serum creatinine is 0.6 - 1.5 mg / dl. The glomerulus filters creatinine, and thus, serum creatinine levels are considered an indirect measure of glomerular filtration. The damage to the glomerulus leads to decreased glomerular filtration rate results in a rise in the concentration of creatinine in serum. The increase indicates the progression of kidney disease, and so serum creatinine has a more remarkable predictive ability than urea for predicting the adverse outcomes.[16]

The mean value of blood urea was high in CRF patients than in healthy controls. ($P < 0.0001$). Our findings are also similar to previous studies by SN Sridhar AV et, 2 Pandya et al.[16] The average blood urea concentration is 20 - 40 mg / dl. The diminishing of the glomerular filtration rate will result in a rise in blood urea concentration.[16] In our study, the mean serum MDA in chronic renal failure patients is high compared to control ($p < 0.0001$). The obtained results were consistent with previous studies by Nagane NS et al,[5] SN Sridhar AV et al,[2] Lakshmi SB et al.[15] A significant positive correlation existed between serum MDA and serum creatinine ($r = 0.4683$).

CRF is a pro-oxidant state that the degree of intracellular and extracellular oxidative stress is mostly related to the severity of the renal failure. The oxidative stress plays an essential role in chronic kidney disease: the oxidative state is thought to be a state of alteration between the generation of free radicals and their degradation by antioxidant systems. If not balanced, this leads to increased accumulation of the radicals, is known to occur in patients with CKD and further contributes to inflammation, endothelial dysfunction, risk of atherosclerosis and progression of CKD. Several pro-oxidant factors, including advanced age, diabetes, and renal hypertension, low levels of antioxidant vitamins, uraemia-related factors and dialysis-related factors, contribute to the oxidative stress in CKD patients.[2] Polyunsaturated fatty acids are always highly susceptible to damage done by free radicals. The free radical-induced peroxidation of membrane lipids usually occurs in three stages-initiation, propagation and termination.[8] The pathology of oxidative stress in acceleration of cardiovascular disease pathogenesis in CKD patients is well-known.

The cardiovascular-related disease is still one among the leading causes of mortality in patients with chronic kidney disease. Mortality due to cardiovascular diseases in patients with end-stage renal disease is 10 to 20 times higher than in the general population. This is due to oxidative stress and inflammation inducing diminished endothelial function and impair vascular structural and functional parameters.

Reactive oxygen species can react with polyunsaturated fatty acids to produce lipid hydroperoxides. MDA is the resultant breakdown product of chain reactions that lead to oxidation of PUFAS. MDA can also interact with DNA and proteins and has mutagenic and cytotoxic effects. They are possibly involved in the pathogenesis of several human diseases, including atherosclerosis. MDA levels increase with the progression of kidney dysfunction. MDA is a useful

biochemical marker for assessing oxidative damage.[16,17] In this present study, the mean value of serum hs-CRP in CRF patients is high compared to healthy controls ($p < 0.001$). Previous studies support our study findings by Nagane NS et al [5], Lakshmi BS et al.[18]

A positive correlation existed between serum hs-CRP and serum creatinine ($r = 0.3010$), serum MDA ($r = 0.02340$). So, the tests of MDA and hs-CRP are positively correlated and may be done to see the extent of inflammation induced oxidative damage. Elevated serum CRP levels are linked with the development of atherosclerosis in CKD patients[19]. The levels of inflammatory markers like CRP are high in coronary heart disease.[20,21] Atherosclerosis and glomerulosclerosis are the most common pathogenesis to many types of kidney damage. CRP reflects the inflammation process involved in glomerulosclerosis. The inflammatory cascade, which is induced by macrophages and monocytes, damage tubular interstitial cells.

In endothelial cells, CRP acts by triggering adhesion molecules to produce interleukin 6 (IL6) and monocyte chemo attractant protein (MCP-1). These could increase the inflammatory level in the plaque by attracting more monocytes and lymphocytes, which in turn induce endothelial damage. The risk factors for kidney function loss, such as obesity, smoking, hypertension, diabetes mellitus, are commonly associated with elevated CRP and inflammatory status.

CONCLUSIONS

Our study shows that Serum MDA levels are increased in states of oxidative stress, in cases of chronic renal failure. Our study also shows that hs-CRP is also increased in cases of CRF. Our study shows a positive correlation between MDA & hs-CRP. The correlation is due to inflammatory damage in renal cells and tissue which may aggravate the oxidative stress. Testing both hs-CRP and MDA will help in early intervention in reducing inflammation and inflammation induced oxidative stress and damage. Early intervention to reduce inflammation will decrease oxidative damage and there by delay in onset of CRF.

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