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Research Article

Oxidative stress markers and inflammatory markers in chronic renal failure

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ABSTRACT

Chronic renal failure is associated with an extensive and complex set of pathological consequences that can result in a number of irreversible, but preventable complications affecting every organ of the body. Chronic renal failure is associated with insulin resistance, hypertension, and increased glycation of proteins, proteinuria, anaemia, cardiovascular diseases and hypothyroidism. Our aim of the study oxidative stress markers and inflammatory markers in chronic renal failure. Methods and Materials: the Present study was done at Fathima Institute of Medical Sciences, Kadapa. Andhra Pradesh. Thirty six chronic renal failure patients, 40 healthy individuals were taken for study. oxidative stress markers and inflammatory markers were estimated by different methods. Conclusion: In our study workprovidesthefirstevidencethatthedecreasedinphosphorylationofIRS -1inresponseto insulin stimulation in animal models of both oxidative stress and CRF could be the mai n culprit in causing insulin resistance. Further, alteration of redox sensitive stress kinases seems to have a major hand in decreasingIRS -1tyrosinephosphorylation.

Keywords: Inflammatory markers; Inter Leakiness; MDA; Oxidative stress markers .

INTRODUCTION

Chronic renal failure (CRF) is silent killer. Chronic Kidney Disease is a growing problem that affects approximately 12% of the adult population. Major risk factors of CRF are diabetes mellitus, hypertension glomerular nephritis, urinary track infection, kidney stones and some drugs. Most chronic nephropathies and renal diseases are lack specific treatment and progress related to CRF prevalence of which is increasing worldwide.

CRF is associated with an extensive and complex set of pathological consequences that can result in a number of irreversible, but preventable complications affecting every organ of the body. Chronic renal disease is associated with insulin resistance, hypertension, increased glycation of proteins, proteinuria, anemia, cardiovascular diseases and hypothyroidism, these disorders have a multifactorial etiology, there is now strong correlative evidence implicating the formation of reactive oxygen species (ROS) and the accompanying increase in oxidative stress as key contributors to these biological perturbation and there by contributing significantly to the progressive decline in renal function in CRF (Locatelli F et al., 2003).

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Oxidative stress is purported to play an important role in the pathogenesis of chronic renal disease. The presence of oxidative stress and inflammation in chronic renal failure has been well established. The consequences of oxidative stress in this pathological condition have not been well elucidated. This knowledge will provide an opportunity to assess the benefit of antioxidant therapy in retarding or preventing there nays function in chronic renal failure patients.

Oxidative stress is assessed either by measuring markers of the oxidative damage to polyunsaturated fatty acids, such as malondialdehyde (lipid peroxides) or by inference from the levels of antioxidants. The antioxidants, which counter the attack of reactive oxygen species include intracellular antioxidant enzymes such as super oxide dismutase (SOD), glutathione peroxidase (GP), and catalase and non-enzymatic chain-breaking antioxidants present in the plasma like vitamin E, A, and C(a).

Cytokines are substances those are playing an important role in coordinating the inflammatory Response of the body to various external and internal stimuli (Romagnani S et al.,2000).Cytokines are classified in two types they are : 1)Pro inflammatory 2) anti -inflammatory (Taniguchi T et al., 1997). The Pro inflammatory cytokines are essential to initiate defence against various pathogenic agents. In certain conditions, there is an overproduction of the pro inflammatory cytokines and the result counterproductive (Pinsky MR et al., 2007). The anti-inflammatory cytokines down regulate the inflammatory process, in part by suppressing production of the pro inflammatory cyto-

kines and therefore help to balance the inflammatory response (Gerard C et al., 1993). Same like as pro inflammatory cytokines, excess secretion of anti - inflammatory cytokines may have deleterious effects on organ function (Bone RC et al.,1996).The anti - inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-10, and IL-13and the pro inflammatory cytokines include interleukin (IL)-1β,IL-2 IL-6, IL-8, and Tumour necrosis factor (TNF)-alpha. Pro inflammatory markers like as C-reactive protein and IL-6 are reliable predictors of CVD in adult dialysis patients (Stenvinkel P et al., 1994).

AIM AND OBJECTS

The objectives of this study were:

1. To investigate oxidative stress markers and inflammatory markers in patients with CRF, comparable healthy controls and to characterize their relationship to renal function.
2. To examine the relationship between markers of oxidative stress and conventional risk markers of cardiovascular risk factors in patients with CRF and to evaluate the possible protective role of antioxidants in animal model.

MATERIALS AND METHODS

Antibodies and reagents

Insulin receptorβ-subunit, IRS–1, IKBα, P-p38MAPK, p38MAPK, P-JNK1, JNK1, IKBα, phosphor tyrosine,β-actin antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). Protein-Aagarose slurry was purchased from Bangalore Genei (Bangalore, India).Xanthine oxidase was procured from (SRL, Mumbai, India). Insulin (human recombinant), sodium molybdate, phenyl methylsulfonyl fluoride, a protein in, leupeptin, okadaicacid, NonidetP-40, sodium deoxycholate and all other chemicals were purchased from Sigma Chemicals (St. Louis, MO). Green tea was procured from local supermarket. Thirty six chronic renal failure patients (24malesand12females) attending the medicine out patients department in Fathima institute of Medical Sciences, Kadapa were included in this study. Ultrasound reports of CRF patients revealed loss of cortico medullary differentiation and they showed clinical signs and symptoms of azotaemia. Diabetic CRF patients and patients who had under gone dialysis previously were excluded from the study. Patients who shows symptom of endocrine disorders associated with hyperglycaemia and those suffering from any acute infection were also avoided. Forty age-matched healthy volunteers (13females and 27males) were enrolled as controls in the present study. Subjects with history of diabetes, renal disease, coronary heart disease, endocrine dysfunction, smokers and alcoholic and those who were on any kind of medication were excluded from the study. All the experimental procedures were approved by the Institute Human Ethics Committee (IHEC) and informed consent was obtained from all the participants.

Blood sample collection

On the day of the study patients reported to our Bio chemistry laboratory in the morning after an overnight fasting of 10-12hours. In all the patients 7ml of venous blood was collected from them in the bottles containing EDTA. Whole blood was used for the estimation of glycated haemoglobin. Plasma was collected by centrifuging the sample at 5000*g* for minutes at 4°C. Plasma glucose, lipid profile, urea and creatinine were estimated immediately the collection and rest of the plasma was stored a -70OC for the estimation offructosamine, IL-6, TNF-alphaandhsCRPand oxidized-LDL.

Analysis of plasma biochemical parameters

Plasma glucose, urea, creatinine was estimated in fasting samples using standard reagent kit adapted to the 550 Express Plus clinical chemistry analyser (Bayers Diagnostics, USA). Fasting plasma insulin was estimated using human insulin ELISA kit following manufacture's (Biol-LineS.A,Belgium)instructions. Plasma fructosamine was measured by p-indonitrotetrazolium violet kinetic method using Raichem kits (Haemagen Diagnostics, San Diego, CA) adapted to 550 express plus Plus clinical chemistry analyser (Bayers Diagnostics, USA). TNF-alphaandIL-6 were estimated using ELISA kit following the manufacture's (Immunotech, Prague, CzechRepublic) instructions. Glycated haemoglobin was measured by using haemoglobin A1C micro columns (Biocon, Vohl-Marienhagen, Germany) and expressed as the percent of total haemoglobin. Oxidized LDL was estimated using ELISA kit following the manufacture's (Mecodia AB, Uppsala, Sweden) instruction. From the fasting glucose and insulin values the homeostatic model assessment-insulin resistant (HOMA-IR) was calculated using the following formula;

$$HOMR - IR = Fasting\ Insulin\ \left(\frac{\mu U}{ml}\right) \times Fasting\ Glucose\ \left(\frac{mM}{22.5}\right)$$

(Pickavance, L.C et al., 2001).

Analysis of oxidative stress parameters

The plasma MDA was estimated by the method of Yagi (Yagi K et al., 1984). The concentration of MDA was calculated using the molar extinction co-efficient (1.56X105) and expressed as mmol/L.

The erythrocyte reduced glutathione content was determined by the method of Beutle and Kelley (Beutler E, Kelley BM. Et al., 1963). The haemoglobin content of the blood was estimated using Drabkin's solution (Clinical Systems, India). The reduced glutathione concentrations were expressed as mg/gHb.

The catalase activity in erythrocytes was estimated by the method of Aebi (Aebi H et al., 1984). Red blood cells (RBC) were separated from the blood by centrifuging at 3500g for10 min at 40C. The decomposition of

H_2O_2 was measured by monitoring the decrease in the absorbance at 240nm for 60seconds. The catalase activity was expressed as rate constant (U/gHb).The glutathione peroxidase enzyme in erythrocytes was estimated by the method of Wendel (Wendel A et al., 1981).Protein carbonyls were measured by using the method of Reznick and Packer (Reznick AZ, Packer L et al 1994).

Plasma free sulfhydryl group was estimated according to the method of Huetal (Hu ML, Louie S et al., 1993). Molar extinction coefficient at 412nm of 13100 was used for the calculation of the results.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Independent samples't' test was used to test the significance of difference in means between study group and controls. For men and women, a student t-test or ANOVA test was used to compare between control and Renal failure participants respectively. A P-value less than 0.05 were considered statistically significant. Statistical analysis was done by using Microsoft Excel and SPSS for windows version 11.5 (SPSS, Inc., Chicago).

RESULTS AND DISCUSSION

The clinical characteristics of the subjects are shown in table 1.As shown in the table, the study groups were well matched for age with their control groups. The test groups shows significantly increased values of creatinine and urea when compared with controls groups ($p < 0.02$).

In CRF patients as shown in table 1. there was a perturbation in oxidant – antioxidant status. Protein carbonyl and lipid peroxide levels were slightly increased in kidney disease patients compared with healthy control subjects ($p < 0.05$). The superoxide radical can initiate lipid peroxidation of fatty acids and can react with nitric oxide to form peroxynitrite radicals. The peroxynitrite radical can also get converted to hydroxyl radical and nitrates. The hydroxyl radicals can in turn perpetuate lipid peroxidation. (b).

The results of insulin resistance and glycated protein levels are shown in table 2. Fasting plasma insulin levels were significantly increased in Chronic Renal Failure patients when compared with control subjects ($p < 0.05$). No significant difference is seen in between the two groups with respect to the fasting glucose levels. There was difference in insulin sensitivity between the two groups as measured by HOMA-IR. Both glycated hemoglobin and fructosamine values were slightly higher in kidney disease patients when compared with the control subjects ($p < 0.05$).

In table 3, in the CRF group, All the parameters [total cholesterol, triglyceride oxidized LDL and LDL cholesterol] levels were increased, while HDL cholesterol levels were decreased when compared with controls ($p < 0.05$).

The results from table 3, indicate that non-diabetic CRF patients who have not been subjected to any dialysis were in a state of low inflammation. The hsCRP levels were slightly increased in CRF patients when compared with control group ($p < 0.05$). The values of both TNF-α and IL-6 were also increases in CRF patients when compared with the control group ($p < 0.03$).

In CRF group, significant correlations of plasma creatinine were found with MDA ($P < 0.001$) and protein carboxylation ($P < 0.002$). A positive associ ation was also observed in between plasma creatinine and glutathione peroxidase ($p < 0.05$) in the test group.

Plasma creatinine also had significant association with inflammatory markers in CRF group. Creatinine had slightly positive correlations with TNF-α ($p < 0.002$), IL-6 ($p < 0.001$) and hsCRP ($p < 0.001$) in CRF group. A slightly positive association was also observed between plasma creatinine and oxidized LDL ($p < 0.001$) in CRF patients. Plasma creatinine had a significant positive correlation with both glycated hemoglobin ($p < 0.05$) and plasma fructosamine ($p < 0.02$) in the test group. Plasma fasting insulin and HOMA-IR also had significant positive correlations with creatinine in CRF group ($p < 0.002$ and $p < 0.002$) respectively.

The inflammatory markers evaluated in the present study had a significant positive correlation with plasma MDA in CRF patients. Plasma MDA had a positive correlation with TNF-alpha ($p < 0.02$), IL-6 ($p < 0.02$) and hsCRP ($p < 0.01$) in the test group. A significant positive association was also observed between plasma MDA and oxidized LDL ($p < 0.001$) in CRF patients.

Plasma MDA had a significant correlation with glycated hemoglobin ($p < 0.002$) and fructosamine ($p < 0.001$) in the test group. Plasma MDA also had a positive correlation with fasting insulin ($p < 0.006$) and HOMA – IR ($p < 0.01$) in CRF group.

Erythrocyte glutathione peroxidase had slightly negative correlation with reduced glutathione ($r = -0.34$; $p < 0.05$) in test group. Superoxide dismutase, glutathione peroxidase and catalase, together with glutathione, form the main line of defense against ROS in erythrocytes. The activity of glutathione peroxidase was significantly increased in CRF patients when compared with control groups ($p < 0.05$).There was difference in erythrocyte catalase activity between the two test groups (c).

Plasma protein carbonyl had a positive correlation with insulin ($r = 0.40$; $p < 0.01$) and HOMA-IR ($r = 0.43$; $p < 0.01$) in CRF patients.

Table 1: Biochemical and oxidative stress parameters of patients with chronic renal failure and age-matched healthy control subjects

Parameter	Control subjects (n = 40)	CRF patients (n = 36)
Age (years)	36.65 ± 7.27	34.72 ± 7.94
Urea (mg/dl)	27.08 ± 5.32	122.32 ± 49.67 *
Creatinine(mg/dl)	0.82 ± 0.25	5.79 ± 3.18 *
Lipid peroxides (nmol/L)	1.56 ± 0.40	2.89 ± 0.51 *
Protein carbonyl (nmol/mg protein)	1.84 ± 0.52	2.57 ± 0.69 *
Free sulfhydryl group (nmol/L)	474.75 ± 74.87	374.87 ± 65.91 *
Erythrocyte reduced GSH (mg/g Hb)	3.51 ± 1.27	2.18 ± 1.27 *
Erythrocyte glutathione peroxidase (U/g Hb)	42.69 ± 24.85	93.43 ± 40.34 *
Erythrocyte Catalase (U/g Hb)	23.20 ± 11.86	18.67 ± 7.72

Data are expressed as mean ± S.D. '*' P < 0.05 compared to controls subjects

Table 2: Plasma glucose, insulin, HOMA-IR and protein glycation levels in CRF patients and age-matched healthy control subjects

Parameter	Control subjects (n = 40)	CRF patients (n = 36)
Plasma fasting glucose (mg/dl)	86.10 ± 9.04	83.42 ± 10.80
Plasma fasting insulin (pmol/l)	21.37 ± 9.77	38.44 ± 12.29 *
HOMA-IR	4.17 ± 0.26	8.52 ± 3.03 *
Glycated hemoglobin (%)	5.56 ± 0.87	6.81 ± 0.80 *
Fructosamine (mmol/L)	2.03 ± 0.42	2.84 ± 0.69 *

Data are expressed as mean ± S.D. '*' P < 0.05 compared to controls subjects.

Table 3: Lipid profile and levels of inflammatory markers in chronic renal failure and age-matched healthy control subjects

Parameter	Control subjects (n = 40)	CRF patients (n = 36)
Total cholesterol (mg/dl)	165.00 ± 24.82	20.34 ± 28.65
Triglyceride (mg/dl)	98.53 ± 51.03	165.66 ± 66.56
HDL cholesterol (mg/dl)	48.73 10.92	43.72 ± 12.12
LDL cholesterol (mg/dl)	95.35 ± 27.22	126.81 ± 29.86
VLDL cholesterol (mg/dl)	19.93 ± 9.90	34.33 ± 13.36
Oxidized LDL (U/L)	35.55 ± 9.31	72.47 ± 12.75
TNF - alpha (pg/ml)	2.15 ± 0.69	4.54 ± 3.04
IL - 6 (pg/ml)	2.35 ± 0.70	5.14 ± 4.47
hsCRP (mg/dl)	0.84 ± 0.22	2.84 ± 0.58

Data are expressed as mean ± S.D. * P < 0.05 compared to controls subjects

Table 4: Correlation of creatinine, oxidative stress parameters and inflammatory markers with plasma insulin and HOMA-IR in CRF patients (n = 36).

Parameter	Plasma insulin		HOMA-IR	
	r	p	r	p
Creatinine	0.51	< 0.002	0.53	< 0.002
Plasma MDA	0.48	< 0.006	0.44	< 0.01
Protein carbonyl	0.50	< 0.005	0.39	< 0.03
Glutathione peroxidase	0.40	< 0.01	0.43	< 0.01
TNF – alpha	0.62	< 0.001	0.66	< 0.001
IL – 6	0.48	< 0.006	0.39	< 0.03
hsCRP	0.53	< 0.005	0.56	< 0.001
Oxidized LDL	0.51	< 0.005	0.53	< 0.005

Reduced glutathione had a significant negative correlation with glycated hemoglobin ($p < 0.005$) and fructosamine ($p < 0.01$) in test group. A significant negative correlation was also observed between reduced glutathione and hsCRP ($p < 0.05$).

In CRF patients a significant correlation was also observed between inflammatory markers with plasma insulin and HOMA-IR. Plasma fasting insulin had a significant positive association with TNF-alpha ($p < 0.001$), IL-6 ($p < 0.002$) and hsCRP ($p < 0.005$) in the test group. A significant positive correlation was observed

Table 5: Correlation of creatinine, oxidative stress parameters, TNF - alpha and oxidized LDL markers with glycated hemoglobin and fructosamine in CRF patients (n = 36)

Parameter	Plasma insulin		HOMA-IR	
	r	p	r	p
Creatinine	0.32	< 0.05	0.41	< 0.02
Plasma MDA	0.51	< 0.002	0.60	< 0.001
Reduced glutathione	- 0.51	< 0.002	- 0.43	< 0.01
TNF – alpha	0.48	< 0.001	0.59	< 0.001
Oxidized LDL	0.33	< 0.05	0.40	< 0.05

Table 6: Correlation of oxidative stress parameters and inflammatory markers with oxidized LDL and creatinine in CRF patients (n = 36)

Parameter	Oxidized LDL		Creatinine	
	r	p	r	p
Plasma MDA	0.57	< 0.001	0.65	< 0.001
Protein Carbonyl	0.39	< 0.03	0.53	< 0.001
TNF – alpha	0.48	< 0.002	0.52	< 0.001
IL-6	0.51	< 0.002	0.63	< 0.001
hsCRP	0.56	< 0.001	0.62	< 0.001

between HOMA-IR with TNF-alpha ($p < 0.001$), IL-6 ($p < 0.03$) and hsCRP ($p < 0.003$) in CRF patients. Oxidized LDL also had a significant positive correlation with fasting insulin ($p < 0.005$) and HOMA-IR ($p < 0.005$) in the test group.

Both glycated hemoglobin and plasma fructos amine had a positive association with TNF-alpha ($p < 0.01$ and $p < 0.005$). Similarly both glycated hemoglobin and plasma fructosamine had a slightly positive association with oxidized LDL ($p < 0.05$ and $p < 0.05$) in CRF patients.

Oxidative stress and inflammatory status has become an important one innumerous fundamental and clinical. ROS are highly reactive and are capable of damaging every part of the cell and every biomolecule in the living system. Oxidation and reduction modulate all cellular events, including modification of signalling molecules in intra- and inter cellular communication (Pamplona R, Prat J et ai., 1996).

In the present study CRF patients who were non-diabetic and have not undergone any dialysis are in a state of severe oxidative stress. This perturbation in redox status was related to type of renal failure. There was a significant relationship found in between oxidative stress and inflammation in CRF patients. Further, there was a significance association between the degree of oxidative stress (as estimated by plasma MDA and carbonyl) with fasting hyper insulinemia and insulin resistance. Significant association was also observed between the inflammatory parameters with HOMA-IR. These findings indicates that insulin resistance in CRF are due to both oxidative stress and inflammation components. There was a significant association between oxidative stress and the process of protein glycation in CRF patients. The in vitro studies have also strengthened the hypothesis that oxidative stress can enhance the process of protein glycation. Further, lipoic acid, taurine and green tea extract have been found to prevent the oxidative stress induced glycation of hemoglobin. A close association was observed between oxidative stress, inflammation and ox-LDL in CRF patients, suggesting that this unholytriad can drive these patients into atherosclerotic complications if left unchecked.

Further significant protective effect against oxidative stress and insulin resistance were evident in control rats supplemented with both green tea and taurine. The supplementation of control rats with green tea or taurine was associated with decreased activation of redox sensitive serine kinase pathways (NF-k Band JNK), and increased insulin stimulated IRS-1tyrosine phosphorylation.

Studies in the past have demonstrated the pathophysiological importance of the metabolites of partially reduced oxygen molecules or reactive oxygen species (ROS) in various experimental renal diseases, including several animal models of primary glomerulopathy and acute renal failure, both ischemic and nephrotoxic (Shah SV et al., 1989). Accumulating evidences from clinical studies have also suggested that CRF is associated with enhanced oxidative stress (Himmelfarb J, Mc Monagle E, et al., 2000).

The pentose phosphate pathway supplies NADPH required by GR to generate reduced Glutathione. It is known that this pathway is impaired in the uremic state leading to a decreased content of reduced Glutathione in erythrocytes which is required for GPX activity. The increased GR activity suggests that there may be a transient improvement in the pentose phosphate pathway because of improvement in the uremic state due to dialysis.

CONCLUSION

Knowledge about the cellular mechanisms responsible for insulin resistance and endothelial dysfunction in chronic renal failure is of clinical and scientific relevance to identify targets for the development of novel therapeutics to treat CRF. In our study describes the mechanisms by which oxidative stress could induce insulin resistance in CRF. In our study work provides the first evidence that the decreased in phosphorylation of IRS-1in response to insulin stimulation in animal models of both oxidative stress and CRF could be the main culpritin causing insulin resistance. Further, alteration of redox sensitive stress kinases seems to have a major hand in decreasing IRS-1tyrosine phosphorylation. In addition, we have identified new strategies to prevent insulin resistance by use of green tea and taurine. Overall, we can conclude oxidative stress via the activation of stress sensitive kinases (JNK, p38MPAK amdNF-kB) plays an important role in causing insulin resistance in CRF. Therefore, oxidative stress along with stress sensitive kinases should be considered as potential target in treatment of CRF along with conventional therapy.

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