Original Article

Study on tumour necrosis factor alpha (α) and insulin resistance in chronic kidney disease

Lakshmi D[1*], K S Meera[2], Mahesh E[3]

{[1]PG. Student, [2]Professor, Department of Biochemistry} {[3]Professor, Department of Nephrology}
M. S. Ramaiah Medical College, MSR Nagar, MSRIT Post, Bangalore, Karnataka-560054, INDIA.
Email: drlakshmimurthy@gmail.com

Abstract

Aim: The study is intended to know the levels of Tumour Necrosis Factor α, an inflammatory marker, Fasting insulin level and insulin resistance in chronic kidney disease per se. **Materials and Methods**: The study population included 45 CKD cases and 45 healthy controls of either gender. Fasting blood glucose, Blood urea nitrogen, S. creatinine, uric acid and hemoglobin were measured. The Fasting Insulin levels and Serum TNF α levels were estimated by ELISA method. eGFR was calculated using MDRD formula and IR was calculated by HOMA-IR index. **Results:** The study shows significant rise in fasting insulin, HOMA-IR and TNF α levels ($p<0.001$) in CKD cases with no significant alteration in fasting blood sugar levels. The correlation of IR with TNF α in different stages of CKD was not found to be significant. **Conclusion**: There is increase in TNF-α in CKD as cytokines can interfere with insulin signaling mechanism and can predispose to vascular wall inflammation, endothelial dysfunction and subsequently to atherogenesis. Hyperinsulinemia, increased resistance to insulin action associated with normoglycemia in the present study indicates pancreatic β cells are functioning normally. No significant correlation between IR and TNF-α highlight their role as independent factors.
Keywords: Atherogenesis, chronic kidney disease, endothelial dysfunction, inflammation, insulin resistance.

[*]**Address for Correspondence:**
Dr. Lakshmi D, Department of Biochemistry, M.S. Ramaiah Medical College, MSR Nagar, MSRIT post, Bangalore- 560054, Karnataka, INDIA.
Email: drlakshmimurthy@gmail.com
Received Date: 24/09/2015 Revised Date: 02/11/2015 Accepted Date: 30/12/2015

Access this article online

Quick Response Code:

Website: www.statperson.com

DOI: 01 January 2016

INTRODUCTION

Chronic kidney disease is emerging to be an important chronic disease globally and about 6% of the chronic kidney disease (CKD) cases are reported after stage 3 of CKD[1]. The major outcomes of chronic kidney disease include loss of renal function leading to kidney failure and development of cardiovascular disease. 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]. The atherosclerotic process which is accelerated in renal disease cannot be completely reasoned out by the traditional cardiovascular risk factors like diabetes mellitus, hypertension, dyslipidemia, obesity, smoking and others. The nontraditional cardiovascular risk factors particularly relevant to chronic kidney disease includes inflammation, malnutrition, low serum albumin, anaemia, hyper homocysteinemia, elevated fibrinogen, derangement of calcium and/ or phosphorus metabolism[3]. However, the complex interaction of the nontraditional risk factors in chronic kidney disease is not well understood. Kidney failure is the most discernible outcome of chronic kidney disease and is subsequently associated with complications in virtually every organ system. The mortality and morbidity due to cardiovascular complications are more common in patients with chronic kidney disease than due to renal failure per se[4]. Cardiovascular complications in patients with chronic kidney disease are treatable and potentially avertable. Patients with CKD are found to be associated with increased risk for diabetes mellitus, cardiovascular disease, metabolic syndrome and others. Insulin Resistance has been an underlying cause of type 2 diabetes mellitus and arteriosclerotic vascular disease. Diabetic individuals and individuals with metabolic

How to site this article: Lakshmi D, K. S. Meera, Mahesh E. Study on tumour necrosis factor alpha (α) and insulin resistance in chronic kidney disease. *International Journal of Recent Trends in Science and Technology.* January 2016; 17(3): 154-162. http://www.statperson.com (accessed 02 January 2016).

syndrome are at increased risk for the development of cardiovascular disease. But there is sparse data on the relationship between Insulin resistance, compensatory hyperinsulinemia and risk of progression of CKD in nondiabetics. In CKD, the combination of impaired immune response coupled with persistent immune stimulation and disturbed cytokine network plays a predominant role in low grade systemic inflammation which mediates the process of monocyte influx, proliferation of macrophages and matrix expansion resulting in glomerular sclerosis and tubulointerstitial injuries[5]. These macrophages can promote the production of pro inflammatory cytokines like C reactive protein, interleukin[6], Tumour Necrosis Factor α and others The study is intended to know the levels of Tumour Necrosis Factor α, an inflammatory marker and to decipher if there is any relationship between inflammatory marker and insulin resistance with the progression of CKD. The study would also help us to substantiate the prevalence of insulin resistance in chronic kidney disease per se due to causes other than diabetes mellitus.

MATERIALS AND METHODS

The study was conducted at M.S. Ramaiah Medical College, Bangalore after ethical clearance was obtained from the ethical review board of the institution and informed consent was taken from the study population before the collection of the sample The study population included 45 clinically diagnosed cases of chronic kidney disease who were attending the outpatient clinic of the Department of Nephrology and 45 healthy individuals as controls who come for routine health check up to M.S. Ramaiah Hospitals and were willing to be a part of this study. Exclusion criteria for the recruitment of cases included CKD patients with history of diabetes mellitus, who were on medications like anti inflammatory drugs, ACE inhibitors, Angiotensin receptor blockers or on diuretics, known cases of cardiovascular disease, with acute illness or infection, chronic kidney disease patients on dialysis or patients on insulin therapy. The control subjects also had same exclusion criteria as CKD cases. A detailed history, including drug history was taken from the both the groups. Weight, height, blood pressure were recorded and general physical examination was done. Systemic examination was also done and appropriate laboratory investigations were included. Around 5ml of blood sample was collected after overnight fasting of 10-12 hours from control subjects and CKD patients in vacutainers. The sample collected in lavender vacutainer was used for estimation of hemoglobin as early as possible. The sample collected in yellow vacutainer was allowed to clot and centrifuged at 5000 rpm. The serum was separated at the earliest and used for the estimation of fasting blood sugar, blood urea nitrogen (BUN), serum creatinine, uric acid, fasting Insulin and TNF –α levels. Blood glucose by hexokinase, Serum creatinine by alkaline picrate, kinetic rate blanked, traceable to IFCC-IDMS standardized, BUN by urease-GLDH method and uric acid by uricase method in Roche cobas 6000**.** Hemoglobin concentration is estimated in whole blood by the cyanide free- sodium lauryl sulphate method. The quantitative determination of serum fasting Insulin levels was carried out by DRG Insulin ELISA kit by DRG Instruments GmbH (Germany). They are based on the so-called heterogeneous sandwich method and are carried out in solid phase[6]. The quantitative determination of serum TNF-α was carried out by the Diaclone TNF-α ELISA kit[7]. This is based on Sandwich enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody sets, Streptavidin-horseradish peroxidase (HRP) conjugate, and recombinant cytokines as the standard were used. The plate was read at 450 nm in an ELISA reader (CPC diagnostics, Stat Fax 4700, microstrip reader). Estimated GFR (eGFR) was calculated by using the Modification of Diet in Renal Disease (MDRD) Formula[8].

eGFR (mL/min/1.73 m2) = 175 × (S.cr)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 if African American).

CKD patients were grouped based on eGFR calculated by MDRD formula:

Stage I CKD - eGFR : ≥90mL/min with demonstrable kidney damage
Stage II CKD - eGFR : 60-89 mL/min
Stage III CKD - eGFR : 30-59 mL/min
Stage IV CKD - eGFR : 15-29 mL/min
Stage V CKD - eGFR : <15mL/min

IR is calculated by using Homeostasis Model Assessment (HOMA) model [9].

HOMA-IR = [Fasting insulin (μU/ml) × Fasting glucose (mmol/l) ÷ 22.5.

Statistical Methods

The results were expressed as mean ± SD. Significance was assessed at 5% level of significance. Categorical data was represented in the form of number (n), percentage (%) frequencies and proportions. Student "t" test (two tailed, independent) and Chi-square 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 12.0 software.

RESULTS

The study was undertaken to find insulin resistance and tumor necrosis factor-α levels in chronic kidney disease and compare it with controls.

Table 1: Demographic and anthropometric measurements of CKD cases and controls (mean±SD)

Profiles	Cases	Controls	P value
Age (years)	36.1±9.5	35.1±8.7	0.605
Height (cms)	163.5±7.4	164.5±6.8	0.509
Weight (kgs)	64.3±10.6	65.2±10.3	0.692
BMI (Kg/m^2)	24±3.6	24±2.8	0.977
Waist circumference (cm)	73.6±11.6	78±13.2	0.096
Hip circumference (cm)	82.4±12.2	86.2±13.8	0.178
Waist Hip Ratio	0.9±0.1	0.9±0.0	0.207

In the present study there was no significant difference between CKD cases and controls with respect to Age, Height, Weight, BMI, waist circumference, Hip circumference and Waist hip ratio (Table 1). The age of the study population varied between 25-50 years of age.

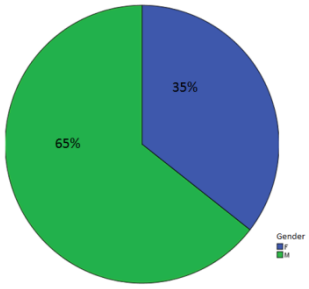

Figure 1: Gender distribution among CKD cases

In the study, it was observed that 65% of CKD cases are males, indicating high prevalence of disease in males as compared to females (Fig 1).

Table 2: Comparison of Biochemical parameters among CKD cases and controls (mean±SD)

Biochemical Parameters	CKD cases (mean±SD)	Controls (mean±SD)	P value
Fasting Blood Glucose (mg/dl)	93.9±17.9	90.5±10.5	0.276
Serum Creatinine (mg/dl)	7.9±5.7	0.8±0.2	<0.001*
Serum Uric Acid (mg/dl)	7.7±2.5	4.5±1.5	<0.001*
Blood Urea Nitrogen (mg/dl)	62.1±35.5	8.6±4	<0.001*
Hemoglobin (gm%)	9.2±2.3	13.6±1.9	<0.001*

In the present study there was no significant difference in Fasting Blood Sugar levels between cases and controls because history of Diabetes mellitus were included as the exclusion criteria (Table 2).

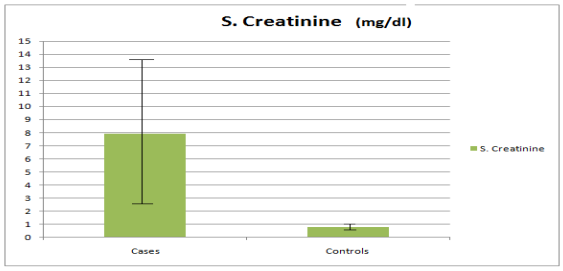

Figure 2: Bar Diagram showing Serum Creatinine levels (mg/dl) in CKD cases and controls (mean±SD)

The serum creatinine level of both the groups is shown in fig 2. In CKD, due to compromise in renal functions there is increase in serum concentration of creatinine. The blood urea nitrogen levels of both the groups are shown in table 2. In CKD, due to compromise in renal functions there is retention of nitrogenous wastes and hence increase in serum concentration of blood urea nitrogen. As evident from the above table 2, there is significant increase in mean values of serum creatinine, uric acid and BUN ($p < 0.001$**). There was significant decrease in Hemoglobin concentration (gm %) in CKD cases when compared to controls ($p < 0.001$) (table 2). Erythropoietin is secreted by peri-tubular fibroblasts and renal failure decreases the production of hemoglobin resulting in anemia.

Table 3: Serum Fasting Insulin levels (µIU/ml), HOMA-IR and TNF-α in CKD cases and Controls (mean±SD)

Biochemical Parameter	CKD cases	Controls	P value
Fasting Insulin (µU/mL)	18.0±4.1	3.6±1.1	<0.001**
HOMA IR	4.1±0.8	0.8±0.2	<0.001**
TNF- α (pg/mL)	8.64±4.97	2.06±1.34	<0.001**

There was significant increase in serum Fasting Insulin level and HOMA IR index in CKD cases when compared to controls ($p < 0.001$) (Table 3). In cases of renal failure, there is decreased clearance of insulin from kidneys. There is significant rise in S. Fasting Insulin levels, with fasting blood sugar levels within physiological range limits. This suggests that CKD cases without diabetes mellitus are having hyperinsulinemia and subsequently manifestations of IR can be observed.

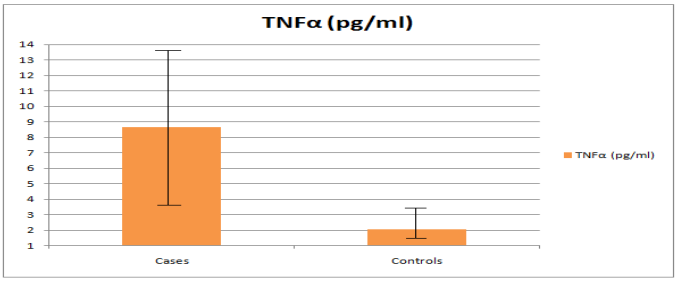

Figure 3: Serum TNF-α in CKD cases and controls. (mean±SD)

It is observed that there is a significant increase in serum TNF-α in CKD patients when compared to controls (Fig 3). Increased production from activated monocytes and decreased excretion from the diseased kidneys subsequently leads to increased levels of serum TNF-α.

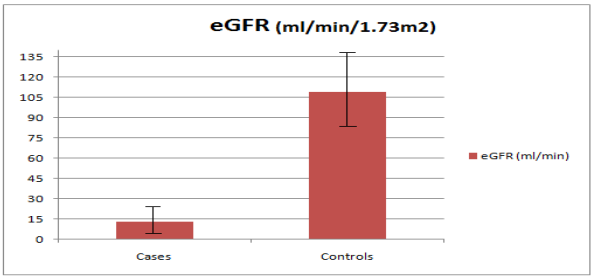

Figure 4: Bar diagram showing eGFR in CKD cases and controls. (mean±SD)

In the study eGFR was 13.24±8.84 ml/min/1.73m^2 in CKD cases and 109±27.96 ml/min/1.73m^2 in controls (fig 4). There is significant decrease in eGFR in CKD cases as compared to controls. As CKD progresses, there is fall in Glomerular filtration rate resulting in decrease in the clearance of the substances.

Table 4: HOMA IR Score in different stages of CKD

		Insulin resistance in CKD Cases		
		IR score >1		
		No. of cases	% of cases	Mean±SD
CKD grades	Stage 1	3	6.7%	4.4 ± 1.6
	Stage 2	1	2.2%	3.2
	Stage 3	5	11.1%	4.4±0.6
	Stage 4	14	31.1%	3.9±0.5
	Stage 5	22	48.9%	4.1±0.9

All the cases had HOMA IR > 1 (100%). The study population had more number of CKD cases who have reported during the later stages of the disease when the progression of the disease is irreversible (Table 4).

Table 5: eGFR level of cases in different stages of CKD (mean±SD)

CKD stages	N	eGFR(ml/min/1.73m^2) (mean±SD)	P value
Stage 1	3	81.93±27.68	
Stage 2	1	60.00±0.00	
Stage 3	5	42.96±3.31	<0.001**
Stage 4	14	22.71±4.46	
Stage 5	22	9.55±3.56	

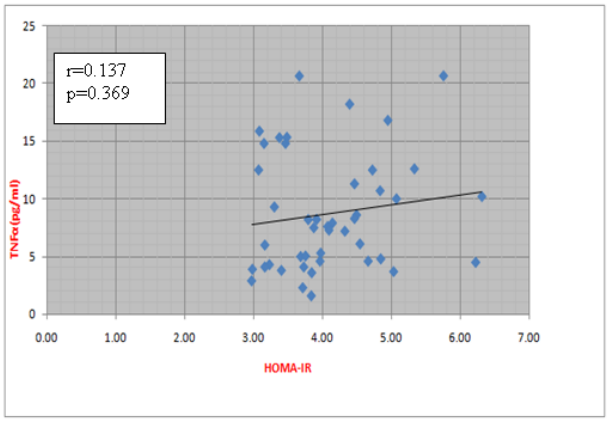

Figure 5: Scatter plot showing relationship between HOMA-IR and TNF-α in CKD cases

In CKD cases there is increase in both TNF alpha and HOMA IR. There was no-significant correlation (r = 0.137) between HOMA IR and TNF – α in CKD cases indicating both parameters are not found dependent on each other in the present study (Fig 5). Insulin resistance and inflammatory marker, TNF α are independent parameters which are recognized as risk factor for developing cardiovascular disease in cases of CKD.

Table 6: Correlation between eGFR and HOMA IR of cases in different stages of CKD

STAGES	eGFR(ml/min/1.73m^2) (mean±SD)	HOMA IR (mean± SD)	No of cases(n)	Correlation coefficient (r)	P value
Stage 1	81.93±27.68	4.4 ± 1.6	3	---	---
Stage 2	60.00±0.00	3.2	1	---	---
Stage 3	42.96±3.31	4.4±0.6	5	-0.06	0.92
Stage 4	22.71±4.46	3.9±0.5	14	-0.55	**<0.05***
Stage 5	9.55±3.56	4.1±0.9	22	-0.21	0.31

In Stage 1 and 2 only three and one CKD cases could be reported and hence the data was found to be insufficient to study correlation. Stage 3 showed negative correlation between eGFR and Insulin resistance. There was a negative correlation between eGFR and IR in Stage 4 and was statistically significant. There was no significant correlation between these parameters in stage 5(Table 6).

Table 7: Correlation between eGFR and TNFα of cases in different stages of CKD

STAGES	eGFR(ml/min/1.73m$^{2)}$ (mean±SD)	TNF – (pg/ml)(mean±SD)	No of cases (n)	Correlation coefficient (r)	P value
Stage 1	81.93±27.68	4.35±2.6	3	---	---
Stage 2	60.00±0.00	4.2	1	---	---
Stage 3	42.96±3.31	4.68±3.02	5	-0.78	0.11
Stage 4	22.71±4.46	5.19±2.57	14	-0.62	**<0.05***
Stage 5	9.55±3.56	10.36±5.27	22	-0.52	**<0.05***

As shown in table 7, there is statistically significant negative correlation between TNF-α and eGFR in stage 4 and stage 5. As there is worsening of renal disease there is increased secretion of pro inflammatory cytokines.

Table 8: ROC curve to determine the cut off level of TNF-α (pg/ml) in CKD cases

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR	+PV	-PV	AUC
>4.5	75.56	60.5 - 87.1	95.56	78.8 - 97.5	8.50	0.27	48.6	97.1	0.77

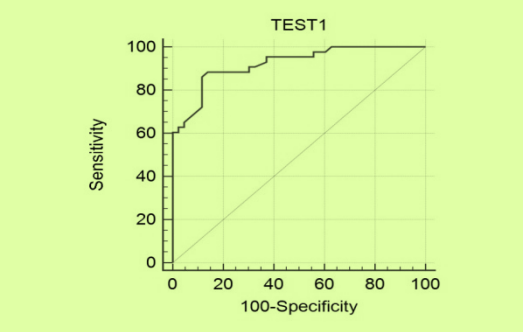

Figure 6: ROC curve to determine the cut off level of TNF-α (pg/ml) in CKD cases

The ROC curve shows that at the cut off value of tumor necrosis factor greater than 4.5 pg/ml, in the present study the sensitivity was about 75.56 and specificity of about 95.56 (Table 8). At values less than the above mentioned cut off level the sensitivity may increase but the specificity comes down (Fig 6). The rise in TNF-α is more specific of CKD as more number of cases had higher level of TNF α in CKD. In the present study, as is evident the CKD cases had high creatinine, blood urea nitrogen and uric acid. On the basis of eGFR, they were divided into different stages. There was hyperinsulinemia and elevated TNF-α level in CKD cases as compared to controls. However no significant correlation was found between Insulin resistance, eGFR and TNF-α with each other in CKD cases. This indicates there is insulin resistance and inflammations in CKD cases, but are found to be independent of each other in the present study.

DISCUSSION

Low grade systemic inflammation is persistently present in CKD and a positive correlation has been found between inflammatory biomarkers levels and decline in GFR[10]. The present study was conducted on non diabetic CKD cases, not on renal replacement therapy or dialysis. The pathophysiology of CKD involves injury to the glomerular basement membrane and tubulointerstitial tissues which leads to decreased excretion of creatinine in urine and elevation in serum creatinine levels. Elevation in serum creatinine level is an indicator of a failing filtration membrane and damaged tubular excretion[11]. In the present study the serum creatinine levels in CKD case was found higher as compared to controls (Table 2). With the progression of the renal dysfunction there is decrease in both glomerular filtration and tubular secretion resulting in the elevation of serum creatinine levels. In the controls, the serum creatinine levels were within the physiological reference interval (Fig 2). In the study populations there was not much of difference with respect to age, height, and hip circumference between CKD cases and controls (Table 1). Though, chronic kidney disease was reported as inflammatory disease of elderly age group but in the present study the mean age of CKD cases was about 36.1±9.5 years and was found to be comparable with the age group of controls (Table 1). This clearly indicates factors like life style, diet, environmental factors have a prominent role to play in the predisposition to renal dysfunction at early age with the modernization of the society. The age group of the study population varied between 25-50 years. Brenner *et al*[12] has reported that in the initial stage of CKD, serum creatinine can be within the physiological limits even when there is considerable loss of nephrons. This can be due to hyperfiltration and increase in intracapillary pressure and GFR in the remaining undamaged nephron. The CKD cases were divided into stages based on eGFR and it was very relevant in the present study the cases were more in stage 3 and above and cases in stage 1 and 2 were very insignificant. In the early stages as mentioned the derangement in S. Creatinine levels would not be very significant. Nearly 65% of the CKD cases were male gender indicating there is gender predisposition. One of the reasons for decreased percentage of female cases may be due to the protective effect of estrogen with high HDL level as most of the females were below 45 years (Fig 1). Although there are gold standard markers available for

estimation of eGFR, Creatinine is used as it is an endogenous substance carrying a low risk of anaphylaxis with a standardized methodology which is IFCC certified and IDMS traceable, thereby carrying high accuracy in eGFR. Estimated GFR (eGFR) is calculated using MDRD formula and the cases were classified into CKD stages based on the eGFR. In the present study it was found that there is gradual decline in eGFR with increased proportion of cases in the later stages (Fig 4). Schiffrin *et al*[4] have reported that in early stages of renal failure there is hyperfiltration which precedes before there is decline in GFR and progresses to overt renal dysfunction without intervention or due to hyperfiltration injury. In the present study it was found that there is increase in Blood urea nitrogen and serum uric acid levels in CKD cases as compared to controls which are suggestive of decreased excretion from the diseased kidneys and also accumulation of uremic toxins (Table 2). Increased accumulation of uremic toxins can interfere with immune defense mechanisms in the CKD subsequently to pre-activation and priming of immune cells leading to inflammation and consequently to CVD[13]. There is no significant difference between mean values of fasting blood sugar of both cases and controls, as the study population were non diabetic individuals (Table 2). TNF-α has been recognized as a pro inflammatory marker which plays a pathogenic role in mediating progressive renal injury. As CKD is associated with low grade inflammation, there is activation of monocytes and macrophages which secrete proinflammatory cytokines. Cachoferio *et al*[14] have reported due to injury to the tissue there is activation of immune cells and production of inflammatory cytokines. Madore *et al*[15] have mentioned, there are multiple causes of inflammation in CKD which includes retention of uremic toxins, sympathetic overactivity and fluid overload. In the present study TNF-α level were found four times elevated in CKD cases as compared to controls (Table 3). There was no evident alteration of TNF-α level between various stages of CKD indicating the rise in TNF-α did not statistically correlate with the decline in renal function (Table 7). The role of other inflammatory markers in CKD may be more predominant like hsCRP, IL-6 and others. IL6 and TNF-α are the pro inflammatory cytokines, but IL 6 has some amount of anti-inflammatory activity also. Mac Donald *et al*[16] have mentioned that proinflammatory cytokines increase the production of reactive oxygen species (ROS) and are themselves regulated in a positive feedback loop via the nuclear factor kappa beta (NFκB) pathway. Nian *et al*[17] have reported the cellular damage caused by the pro inflammatory response stimulates the ROS production cycle and in turn activating further cytokine production. Gupta *et al*[18] have reported TNF-α receptor 2 is found to be positively associated with the risk of developing CKD. It has been reported that albuminuria selectively activates cytokines and elevates TNF-α levels. There is no significant difference between mean values of fasting blood sugar of both cases and controls, as the study population were non diabetic individuals (Table 2). Chronic kidney disease is associated with low hemoglobin level of a mean of about 9.2 ±2.3 gm% as evident from table 2. Hemoglobin values are lower than the biological reference interval indicating the prevalence of anemia. Decreased hemoglobin in CKD is because there is reduced Erythropoietin production by the diseased kidneys. Weiss *et al*[19] suggest that inflammatory cytokines interfere with both proliferation and differentiation of erythroid precursors by several mechanisms, including induction of apoptosis, down regulation of erythropoesis Stimulating agents (ESA) receptors and reduced activity and synthesis of ESAs. Chronic inflammation in CKD is substantiated by increased TNF-α in CKD interferes with the synthesis of erythropoietin. The pro inflammatory cytokines also have a negative impact on other important factors including reduced erythropoietin sensitivity and predispose to anemia in CKD cases. In CKD, there is injury to the renal tissue which synthesizes erythropoietin as a result of which erythropoietin levels are decreased with a consequent increase in hepcidin levels. The synthesis of hepcidin is primarily induced by inflammatory cytokines[20]. In the present study there is fivefold increase in fasting insulin levels in CKD as compared to controls (Table 3) The increase in serum levels of fasting insulin with normal fasting blood glucose levels suggesting that the pancreatic β cells are adequately performing their physiological functions and the resistance offered to the actions of insulin were due to a post receptor defect where there is decreased phosphorylation of IRS[21]. It is the ability of the beta cells to modify the rate of insulin secretion that enables individuals with varying degree of insulin resistance to have normoglycaemia. CKD cases were not included for the study if there were on exogenous insulin therapy, thus excluding false positive values of increased serum insulin levels. The HOMA-IR was found more than three time increase in CKD cases as compared to controls (Table 3). One of the reasons for higher fasting insulin levels in CKD may be due to decreased clearance of insulin by the kidney[22]. The mean values of IR in cases were high when compared to controls and was highly significant. There is hyperinsulinemia associated with high HOMA-IR but with normal glycemia indicates the amplified capacity to withstand the derangement by the pancreas and liver (Table 3, 2). Hyperinsulinemia is a compensatory response to the insulin resistance. The study also showed

a statistically significant association between insulin resistance and decline in eGFR in stage 4 of CKD (Table 6). The reason for not getting significant correlation between IR and eGFR in CKD cases may be due to smaller sample size and due to single estimation of IR. Kobayashi *et al*[23] have reported IR to be common even in patients with mild-to-moderate CKD. Patients with higher insulin resistance had significant alterations in IGF-1 pathway activity. IGF is a key peptide involved in cell growth and protein turn over acting as the primary mediator of many responses regulated by GH in tissues. Alteration in IGF-1 pathway increases the catabolic activity of an individual. The suppression of insulin signaling via phosphorylation of the insulin receptor substrate (IRS1) due to activation of c-Jun N-terminal kinase (JNK) plays an important role. Downstream function of the IRS-PI3K-Akt pathways was associated with chronic inflammation, metabolic acidosis, vitamin D and parathyroid hormone status, anemia, uremic toxins and adipokines. The predisposing factors responsible for IR in the absence of diabetes mellitus (DM) or obesity in CKD are unknown, but are probably related to factors that contribute to vascular disease, such as inflammation and oxidative stress as observed by Banerjee at al[24]. Adiponectin secreted by the adipose tissue is considered to be an important modulator of insulin sensitivity and its concentration is reduced in patients with CKD suggesting it also as an anti- inflammatory factor. De Fronzo *et al*[25] have reported that collagen synthesis is augmented by insulin and insulin like growth factors. These factors also cause cells to proliferate and contribute to the atherosclerotic process. Within the body once the compensatory mechanism for insulin resistance sets into action a series of events that play an important role in the development of cardiovascular manifestation. HOMA IR, a calculated parameter based on fasting glucose and Fasting insulin levels is a limited surrogate marker because it accounts for <40% of the variability as quantified by more direct measures of insulin resistance[26]. Emoto *et al*[27] have mentioned there is an inverse linear correlation between IR by euglycemic hyperinsulinemic clamp and log-transformed IR by HOMA. Therefore, IR by HOMA can be used as an alternative method to assess IR in patients with renal failure. The HOMA –IR score was found to be greater than 1 in all the CKD cases (Table 4). There is clear indication of prevalence of higher HOMA-IR in CKD case indicating due to the onset of renal dysfunction associated with the decrease in glomerular filtration rate there is disturbance in the insulin metabolism leading to hyperinsulinemia and post receptor defect resulting in insulin resistance. Lumeng *et al*[28] have reported due to the release of cytokines there is predilection towards insulin resistance. There is no significant correlation between eGFR and HOMA IR in various stages of CKD, though there is decline in eGFR and is associated with increased resistance to insulin actions in peripheral tissues [Table 6]. Chen *et al*[29] have reported a greater degree of insulin resistance may predispose to renal injury by worsening of renal hemodynamic. eGFR is negatively correlated(statistically) with TNF alpha levels in stage 4 and 5, but not in the earlier stage [Table7]. There is rise in the level of inflammatory marker, TNF-α with the progression of the renal disease as CKD is a chronic inflammatory disease. The correlation of TNF-α with eGFR was not very significant. As shown in table 7 the correlation between insulin resistance and inflammatory marker, TNF-α in different stages of CKD, was statistically significant. This further implies that these two parameters are independently associated with increased cardiovascular risk in CKD patients. Persistent inflammatory state also contributes to insulin resistance and abnormal endothelial vascular reactivity. TNF-α can mediate both acute and chronic inflammation and is found to be associated with increased CVD morbidity and mortality in general population. TNF-α is considered as the pro-inflammatory mediator introducing the link between inflammation, obesity and IR. IR may be one of the mechanisms through which systemic inflammation may exert its deleterious effect on the cardio vasculature. The detrimental effect of inflammation on vascular disease in CKD may be mediated by vascular unresponsiveness to the action of insulin**.** In case of renal failure cases, endothelial mediated vasodilatation can be impaired due to inhibition of Nitric oxide synthase (NOS) in vascular endothelial cells due to insulin resistance. Insulin resistance and endothelial dysfunction represents early events in individuals at risk for CVD[30]. Increased nitric oxide consumption results from increased levels of ROS and RNS which are generated in greater quantity due to intracellular defects in insulin - mediated glucose and lipid metabolism. Lack of nitric oxide leads to increase in vasoconstriction which contributes towards impaired circulation and tissue ischemia**.** There is increase in sodium retention consequent to hyperinsulinemia and IR in CKD cases. Sympathetic hyper activation and activation of renin-angiotensin-aldosterone system can cause further endothelial dysfunction and vasoconstriction. Reduction in insulin resistance ameliorates endothelial dysfunction and improved tissue sensitivity to insulin improves vascular endothelial function[31]. Inflammation and oxidative stress are evident even in the early stages of CKD. They are known to induce IR primarily via the increased production of proinflammatory cytokines. The study shows increased prevalence of insulin resistance in CKD

and has a pivotal role to play in the declining renal function. The etiologies for IR are multifactorial and are found to be associated with a complex network of chronic inflammation, malnutrition and anemia. TNF-α induces IR through direct or indirect mechanisms. TNF-α can induce IRS-1 serine phosphorylation through activation of serine kinases including IKKβ, c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), protein kinase C (PKC), Akt (PKB)[32]. As in the present study TNF-α is raised in the CKD, with a cut off of 4.5 pg/ml as per ROC curve and the area under curve was found to be 0.77 indicating as a reliable marker. The chronic low grade inflammation plays a predominant role in CKD or the development of micro vascular complication by potentiating endothelial dysfunction. Endothelial dysfunction causes increase in pro-inflammatory cytokines[33]. As the inflammatory markers are raised in all stages of CKD, and persistent inflammatory state further contributes to the abnormal endothelial vascular reactivity. Endothelial dysfunction and elevated circulating levels of inflammatory cytokines have been linked to increased cardiovascular mortality. Inflammation, IR and endothelial dysfunction characterize a key triad for the development and progression of atherosclerosis[34]. The rising inflammation may lead to deteriorating renal function which in turn could lead to further increase in inflammation setting up a vicious cycle. The present study suggests that TNF-α is linked with the severity of CKD independent of other risk factors. The predominance of IR in CKD is found to be independent of impaired glycaemia and obesity signifying the need for greater surveillance, as IR is a modifiable risk factor. Insulin resistance, endothelial dysfunction and inflammation play critical role in the development of atherosclerosis. There are prospect that the detrimental effect of inflammation on vascular disease in CKD may be mediated by vascular unresponsiveness to the action of insulin.

CONCLUSION

The combination of impaired immune response coupled with the persistent immune stimulation may have a role in low grade chronic systemic inflammation and cytokine imbalance in patients with CKD which can predispose to increased cardiovascular risk. The study includes a single measurement of the proinflammatory cytokines which may not represent average levels of these biomarkers overtime. Whether IR is antecedent to CKD or a consequence of impaired kidney function is not very clear. Restoration of normal vascular reactivity, reduction in insulin resistance and attenuation of the vascular inflammatory response can bring about reduction in cardiovascular mortality and morbidity in chronic kidney cases. Further study needs to be carried on larger sample size to assess the effectiveness of therapeutic intervention aimed at improving insulin resistance and towards the inclusion of anti – inflammatory agents to study its efficacy towards improving vascular function which can impede cardiovascular complications in patients with chronic kidney disease.

REFERENCES

1. Singh AK, Farag YMK, Mittal BV, Subramanian KK, Reddy SR, Acharya VN, Almeida AF, Channakeshavamurthy A, Ballal HS, Gaccione P, Issacs R. Epidemiology and risk factors of chronic kidney disease in India – results from the SEEK (Screening and Early Evaluation of Kidney Disease) study. BMC Nephrology. 2013; 14: 114-123.
2. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease a statement from the American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. Circulation. 2003; 108: 2154- 2169.
3. Yamamoto S, Kon V. Mechanisms for increased cardiovascular disease in chronic kidney dysfunction. Curr Opin Nephrol Hypertens. 2009; 18(3): 181–188.
4. Schiffrin EL, Lipman ML, Mann JFE. Chronic Kidney Disease-Effects on the Cardiovascular System. Circulation. 2007; 116: 85-97.
5. Imig JD, Michael J. Ryan MJ. Immune and Inflammatory Role in Renal Disease. Compr Physiol. 2013; 3(2): 957–976.
6. Janiak A, Oset P, Talar-Wojnarowska R, Kumor A, Małecka-Panas E. Insulin, insulin-like growth factor 1 and insulin-like growth factor binding protein 3 serum concentrations in patients with adenomatous colon polyps. Prz Gastroenterol. 2013; 8(5): 308–314
7. Shameem M, Fatima N, Nabeela, Khan HM. Association of TNF-α serum levels with response to antitubercular treatment in MDR tuberculosis patients. Ann Trop Med Public Health 2015;8: 258- 261
8. Level AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and Classification of chronic kidney disease: A position statement from Kidney Disease Improving global outcomes. Kidney International. 2005; 67: 2089-2100.
9. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412-419.
10. Shilipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, Furberg CD, Psaty BM. Elevations of inflammatory and pro coagulant biomarkers in elderly persons with renal insufficiency. Circulation. 2003; 107: 87-92.
11. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. N Am J Med Sci. 2010; 2(4): 170- 173.
12. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease.

Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease NEJM. 1982; 307: 652-659.
13. Cohen G, Hörl WH Immune Dysfunction in Uremia-An Update. Toxins 2012; 4 : 962-990.
14. Cachoferio V, Goicochea M, SG de Vinuesa. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. Kidney Int. 2008; 74: S4-S9
15. Madore F. Uremia-related metabolic cardiac risk factors in chronic kidney disease. Semin Dial. 2003; 16(2): 148-156.
16. Macdonald G, Galley HF, Webster NR. Oxidative stress and gene expression in sepsis. Br J Anaesth. 2003; 90(2): 221 – 232.
17. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodelling. Circulation research. 2004; 94(12): 1543-1553.
18. Gupta J, Mitra N, Kanetsky PA, Devaney J, Wing MR, Reilly M, Shah VO, Balakrishnan VS, Guzman NJ, Girndt M, Periera BG Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. CJASN. 2012; 7(12): 1938-1946.
19. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J med 2005; 352:1011 – 1023.
20. Rossi E. Hepcidin - the iron regulatory hormone. Clinical Biochemist Reviews. 2005; 26(3): 47-49.
21. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action and type 2 diabetes. Science. 2004; 306: 457-461.
22. Robert HM. Insulin and its role in chronic kidney disease. Paediatr Nephrol. 2008; 23(3): 355-362.
23. Kobayashi S, Maesato K, Moriya H, Ohtake T, Ikeda T. Insulin resistance in patients with chronic kidney disease. Am J Kidney Dis. 2005; 45(2): 275– 280.
24. Banerjee D, Recio-Mayoral A, Chitalia N, Kaski JC Insulin resistance, inflammation, and vascular disease in nondiabetic predialysis chronic kidney disease patients. Clinical cardiology. 2011; 34(6): 360-365.
25. DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes care.1991; 14(3): 173-194.
26. Kim SH, Abbasi F Reaven GM. Impact of degree of obesity on surrogate estimates of Insulin resistance. Diabetes care. 2004; 27:1998-2002.
27. Emoto M, Nishizawa Y, Maekawa K, Hiura Y, Kawagishi T, Shoji T, et al. Homeostasis model assessment as a clinical index of insulin resistance in type 2 diabetic patients treated with sulfonylureas. Diabetes care. 1999; 22(5): 818-822.
28. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011; 121(6): 2111-2117.
29. Chen J, Muntner P, Hamm LL, Fonseca P, Batuman V, Whelton PK. Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. Journal of the American Society of Nephrology. 2003; 14(2): 469-477.
30. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. Diabetes metab res rev. 2006; 22(6): 423-436.
31. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction molecular and pathophysiological mechanisms. Circulation. 2006; 113: 1888-1904.
32. White MF. IRS proteins and the common path to diabetes. Am J of Physiol Endocrinol Metab. 2002; 283(3): E413-E422.
33. Bolton CH, Downs LG, Victory JGG, Dwight JF, Tomson CRV, Makness MI, Pinkney JH. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. Nephrol Dial Transplant. 2001; 166: 1189-1197.
34. Schwartz EA, Reaven PD. Molecular and signaling mechanisms of atherosclerosis in insulin resistance. Endocrinology and metabolism clinics of North America. 2006; 35(3): 525-549.

Source of Support: None Declared
Conflict of Interest: None Declared