Relevance of Insulin Resistance in Chronic Kidney Disease

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ABSTRACT

Introduction and Objectives of the Study: *Chronic Kidney Disease (CKD) is a major prevalent disease worldwide associated with low grade systemic inflammation, which predisposes to higher incidence of atherosclerotic complications in CKD. The study is intended to measure Insulin Resistance (IR) in chronic kidney disease and to decipher if there is any relation between IR and various stages of CKD.*

Methodology: *The study population included 45 CKD and 45 healthy controls of either gender. Fasting Blood Sugar (FBS) and Serum Creatinine were measured in the fasting sample collected from both the groups. Estimated Glomerular Filtration Rate (eGFR) was calculated using MDRD formula. The fasting Insulin levels were estimated by Enzyme Linked Immuno Sorbant Assay (ELISA) method and IR was calculated by Homeostasis Model Assessment - Insulin resistance (HOMA IR) index. Regression analysis was used to find out the relationship between IR and other variables.*

Results: *There was a significant increase in fasting insulin levels and IR in CKD cases (4.09±0.83) as compared to controls (0.8±0.2) [p<0.001], with no significant alteration in blood sugar level. A negative correlation was found between IR and eGFR in stage IV of CKD (r = -0.55, p<0.05) but was only statistically significant. Mutivariate regression analysis showed a significant relationship (R^2 =0.56) of IR with eGFR, creatinine, FBS.*

Conclusion: *In the present study there was hyper insulinemia associated with high HOMA-IR index but fasting blood sugar was not deranged. The study indicates causes for IR are multifactorial and greater degree of insulin resistance will predispose to worsening of renal function.*

Key Words: *Atherogenesis, chronic kidney disease, eGFR, hyperinsulinemia, insulin resistance*

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INTRODUCTION

Chronic kidney disease is one of the global health problems with a prevalence of about 17.2% in India[1]. It is a pathophysiologic process with multiple etiologies, resulting in exorable attrition of nephron number and function. CKD patients have reduced life span and substantial proportion of these individuals die from cardiovascular complications[2]. Cardiovascular diseases are attributed to be one of the leading causes of morbidity and mortality in all stages of chronic kidney disease3.

The CKD patients present with a high prevalence of metabolic syndrome (MS) which is associated with high risk for developing Diabetes Mellitus, Cardiovascular disease and a high all-cause mortality[4]. According to the Adult Treatment Panel (ATP) III criteria, MS is identified by the presence of three or more of the following components[5]:

1. Abdominal obesity - waist circumference ≥ 102 cm in men and ≥ 88 cm in women.
2. Serum Triglycerides ≥ 150 mg/dl
3. High Density Lipoprotein (HDL) cholesterol ≤ 40 mg/dl in men and≤ 50 mg/dl in women
4. Blood Pressure(BP) ≥130/85 mmHg
5. Fasting glucose ≥ 110 mg/dl.

Metabolic syndrome is also associated with hyperinsulinemia and insulin resistance. Insulin resistance is found to be associated with inflammation predisposing to increase in cardiovascular morbidity and mortality. The study was intended to determine the prevalence of insulin resistance in chronic kidney disease per se and to find if there is any correlation between Insulin Resistance with BMI in various stages of CKD.

MATERIALS AND METHODS

The study was conducted in M. S. Ramaiah Medical College after obtaining ethical clearance from the Institutional ethical committee. The study population included 45 clinically diagnosed cases of CKD attending Nephrology OPD. The CKD patients who had serum creatinine levels greater than 1.3 mg/dl and were willing to be a part of the study were recruited as cases. Individuals having Diabetes mellitus, hypertension, acute and chronic inflammatory diseases, cardiovascular diseases, acute kidney injury, CKD patients on Dialysis and on medications for other chronic diseases including insulin injection, anti-inflammatory drugs and immune modulators, which can interfere, were excluded from the

study. The control population included 45 subjects who visited hospital for routine health checkup and had normal renal function. Informed written consent was taken from both the study groups.

The relevant clinical history was taken and clinical examination of the patients was performed. Demographic details were collected from CKD cases and controls. Anthropometric measurements were taken and BMI was calculated. About 5ml Fasting venous sample was collected in BD vacutainer with yellow cap which contains gel and clot activators from both the study groups after a period of 12 hours overnight fasting. The samples were centrifuged at 3000 rpm for about 20 minutes and serum was separated at the earliest. Serum Creatinine was estimated by (Jaffe's) Alkaline picrate kinetic rate blanked IFCC-IDMS traceable and Fasting blood glucose by hexokinase method was analysed on a fully automated *cobas*® 6000 analyser. (Roche diagnostics, Basal, Switzerland). eGFR was calculated by Modification of Diet in Renal Disease(MDRD)[6] formula and cases were classified into different stages of CKD. Serum levels of fasting Insulin was estimated by ELISA method and IR was calculated by HOMA-IR formula[7].

$$\text{HOMA-IR} = \frac{\text{Fasting insulin } (\mu U/ml) \times \text{Fasting glucose (mmol/l)}}{22.5}$$

$$\text{eGFR } (mL/min/1.73\ m^2) = 175 \times (\text{Serum Creatinine}(mg/dl))^{-1.154} \times (\text{Age}(yrs))^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$$

Statistical Analysis

The results are presented as mean±S.D and results of the categorical measurements are presented in Number (%). The significance of the study parameter between cases and controls was established using student's't' test. The Correlation between different variables and IR was established using Pearson correlation coefficient. Univariate regression analysis was used to find out the relationship of IR with BMI, FBS and eGFR. Multivariate regression analysis used to find out relationship between these parameters with IR being a dependent variable. Statistical analysis was done using SPSS software and 'p' value of <0.05 is considered significant.

RESULTS

The data of controls having normal renal profile and chronic kidney disease cases are compared with respect to serum creatinine, FBS, serum fasting insulin and calculated parameters like eGFR, HOMA-IR and BMI. Out of 45 cases included in the study, 65% were males and 35% females (Table 1). Mean eGFR, Insulin levels and HOMA IR was statistically increased in cases as compared to controls with a p value of <0.001 (Table 2). There was no difference in BMI and FBS levels between cases and controls (Table 2).

The estimated GFR (eGFR) was calculated based on MDRD formula and CKD patients were grouped into:

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

In the study 6.7% of cases were in stage 1, 2.2% were in stage 2, 11.1% were in stage 3, 31.1% were in stage 4 and 48.9% were in stage 5. (Fig. 1).

Hyperinsulinemia and insulin resistance in the CKD cases, who were in different stages of chronic kidney disease, was highly significant as compared to controls (p <0.001) showing increased insulin resistance as kidney function deteriorates. Pearson correlation among different variables showed no significant relationship between IR and different variables. Univariate regression analysis for IR with different parameters was not significant. However, multivariate regression analysis of IR as dependent variable was applied which showed significant relationship with $R^2$of 0.56 and F value of 0.0011.

Table 1: Gender Distribution of controls and CKD cases

Gender	Controls	CKD Cases
Male	26(57%)	29(65%)
Female	19(43%)	16(35%)
Total	45(100%)	45(100%)

Expressed as number (percentage).

Table 2: Biochemical profiles in Controls and CKD cases

Parameters	Controls (mean±SD)	CKD cases (mean±SD)	P value
S. Creatinine (mg/dl)	0.76±0.16	7.87±5.74	<0.001***
eGFR (ml/min)	109±27.96	13.24±8.84	<0.001***
Fasting Insulin (µIU/mL)	3.58±1.13	18.04 ± 4.07	<0.001***
IR	0.8±0.2	4.09±0.83	<0.001***
FBS (mg/dl)	90.8±10.2	93.33±17.94	0.31
BMI (kg/m^2)	24±2.83	24.02±2.61	0.47

eGFR-Estimated glomerular filtration rate; IR-Insulin resistance; FBS-Fasting Blood Sugar; BMI-Body Mass Index. Significance was tested using student 't' test.

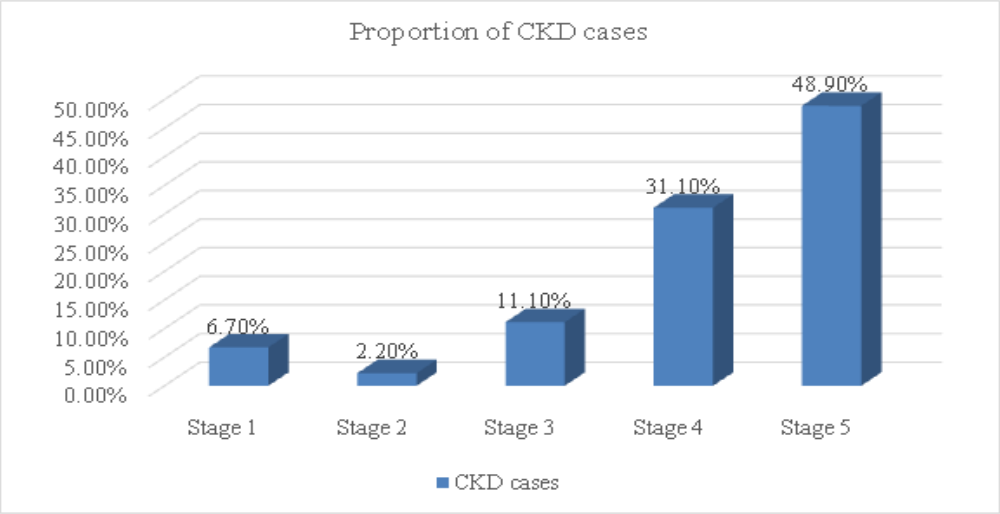

Fig. 1: Bar diagram showing proportion of cases in different stages of CKD

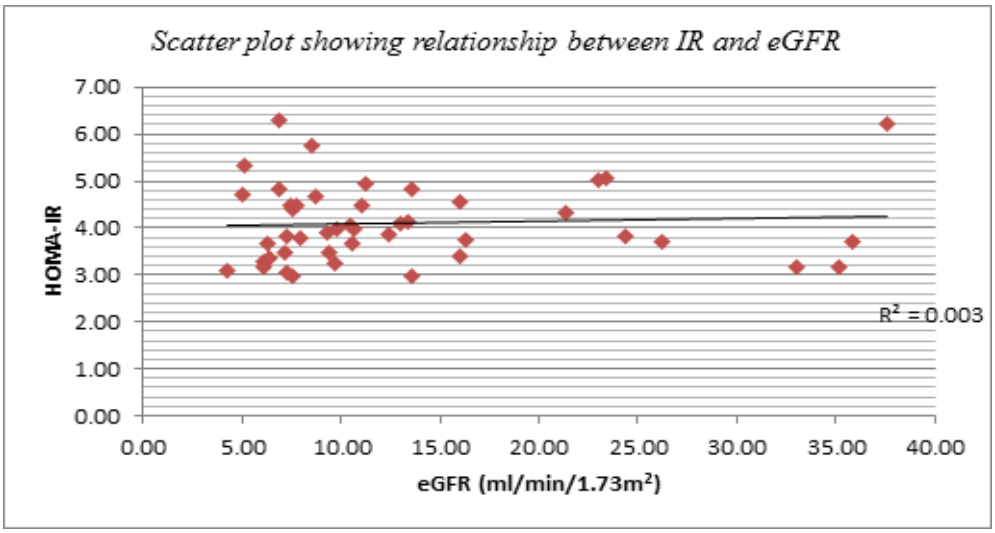

Fig. 2: Scatter plot showing relationship between Insulin resistance and eGFR

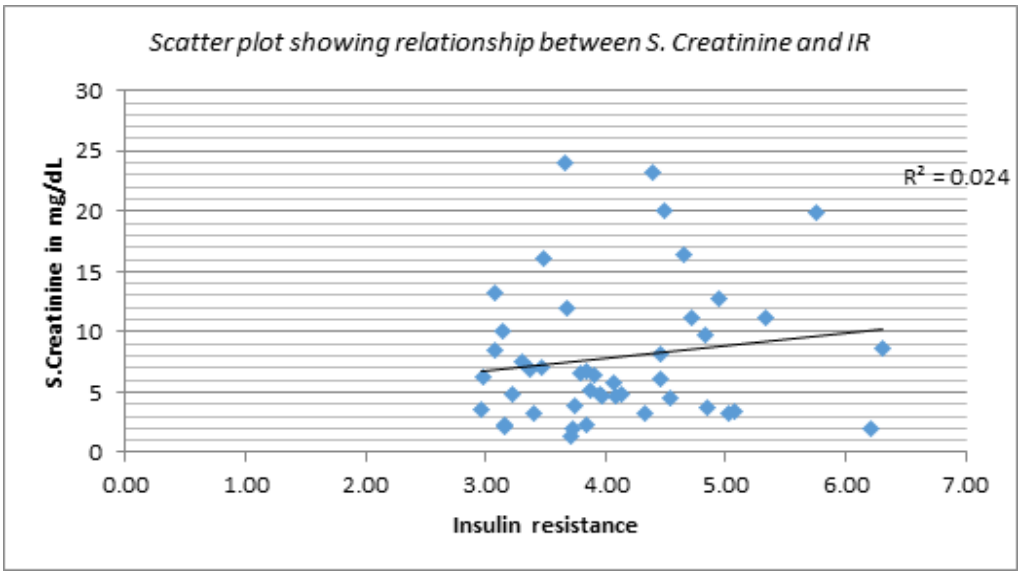

Fig. 3: Scatter plot showing relationship between Insulin resistance and Serum Creatinine

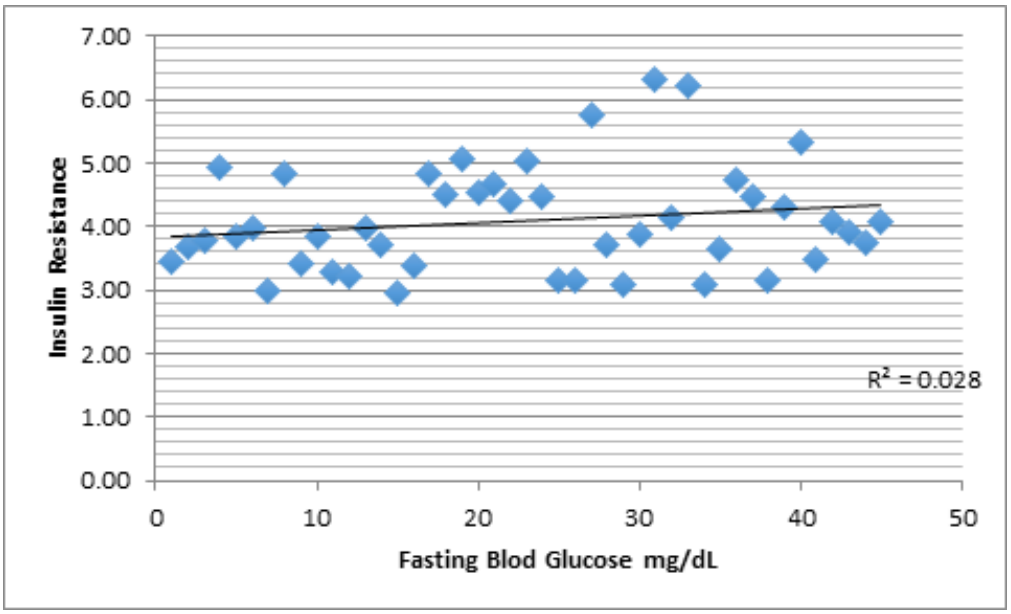

Fig. 4: Scatter plot showing relationship between Insulin resistance and FBS

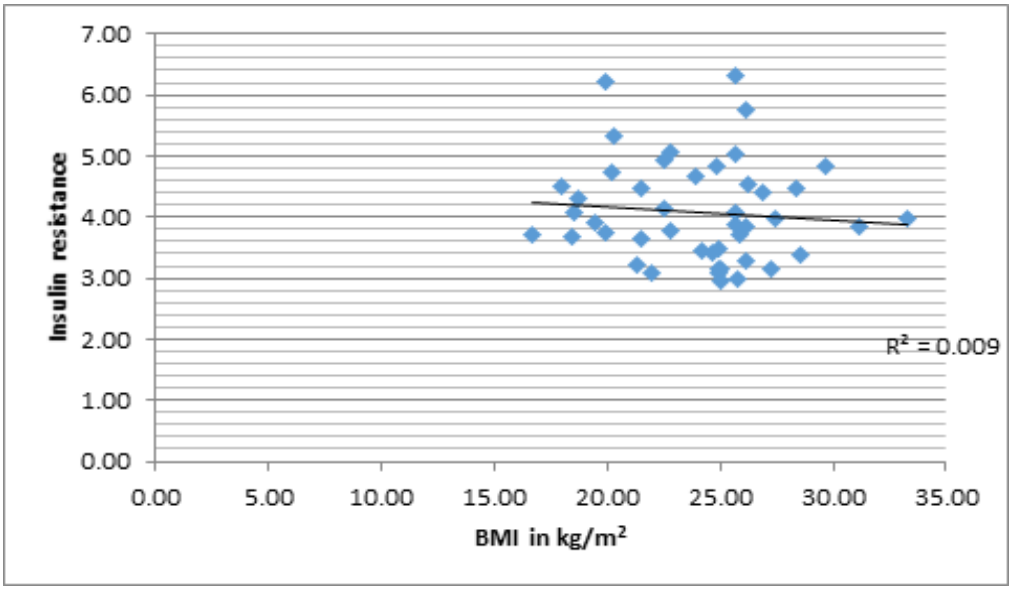

Fig. 5: Scatter plot showing relationship between IR and BMI

Table 3: Univariate regression analysis between IR and other variables

Variables	R^2 (Coefficient of determination)	F value
Fasting Blood Sugar	0.37	0.012
eGFR	0.0001	0.94
Serum Creatinine	0.15	0.30
Body Mass Index	0.009	0.52
Age	0.25	0.08

Univariate regression analysis showing relationship between IR and other variables. FBS can explain only 37% in causing IR. Similarly Creatinine 15%, BMI 9% and age 25%.

Table 4: Multivariate regression analysis with IR as dependent variable

R^2	0.56
Significance F	0.011

DISCUSSION

Chronic kidney disease is associated with alteration in physiologic and metabolic functions ranging from impairment of renal function including deterioration of GFR, along with hyperinsulinemia and insulin resistance as in the present study. In the study the serum creatinine levels was found higher in CKD cases 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 (Table 2). Brenner et al[8] 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 elevation in GFR in the remaining undamaged nephrons.

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). A fivefold increase in fasting insulin levels in CKD cases as compared to controls in the present study (Table 2). The sensitivity of tissue to insulin is remarkably impaired in uraemia and the primary site of the insulin resistance is in peripheral tissue[9]. IR can contribute to the pathogenesis of atherosclerotic cardiovascular complications. Insulin resistance and hyperinsulinemia can act on the proximal tubule to raise blood pressure. Insulin resistance can worsen the inflammatory response in cases and enhance risk of cardio vascular morbidity[10]. The nutritional, metabolic and cardiovascular complications of renal disease may be a consequence of abnormal insulin action. IR is present even in the early stages of renal disease. IR is a component of metabolic syndrome[11]. In the present study there was no significant correlation between IR and BMI because sample size was small and patients with metabolic syndrome were excluded from the study.

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 as cases in stage 1 and 2 were very insignificant (Fig. 1). 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 (Table 1). 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. The progression of metabolic and cellular dysfunction both systemically and locally within kidney tissue is linked to many diverse and complex pathways, which in particularly include heightened production of proinflammatory cytokines[12]. The degree of acidosis has a strategic association with insulin sensitivity. The mechanisms for insulin resistance at different stages of renal failure are diverse because in the present study the study population were heterogeneous. In the study multivariate regression analysis showed a significant relationship between IR and different variables indicating the reason for increase in IR in CKD cases are multifactorial.

Uraemia is characterised by resistance to the action of insulin and is found to be accompanied by hyperinsulinemia as in the present study. Glucose uptake by extra hepatic tissue can be reduced signifying insulin resistance. Insulin resistance and subsequent hyperinsulinemia are involved in the development of atherosclerotic complications. It has been observed when there is subsequent dysfunction of kidney and cardiac system they amplify the progressive failure of both systems[13]. The HOMA-IR was found more than three time increase in CKD cases as compared to controls (Table 2). One of the reasons for elevated fasting insulin levels in CKD may be due to decreased clearance of insulin by the kidney due to the disruption of the function by the kidney[14]. 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 2). Hyperinsulinemia is a compensatory response to the insulin resistance. The study also shows significant rise in insulin resistance and decline in eGFR between CKD cases and controls (Table 2). But Pearson correlation did not show any significant relation between IR and eGFR in CKD cases which may be either due to smaller sample size or due to single estimation of IR. Kobayashi et al[15] have reported IR to be common even in patients with mild-to-moderate chronic kidney disease.

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. IR is an independent predictor of cardiovascular disease and is linked to protein energy wasting and malnutrition. The effects of chronic neuroendocrine stress have a significant effect on down regulating anabolic hormone systems such as the GH-IGF1 axis in humans[16]. Chronic uraemia attenuates GH receptor JAK 2 STAT signal transduction pathways and is characterised by the effects of heightened activities of Hypothalamic Corticotrophin releasing hormone (CRH) and Pituitary Adrenocorticotrophic hormone (ACTH) increasing adrenal cortisol production and induction of Insulin resistance[17].

Insulin resistance is common in patients with mild to moderate stages of CKD even when GFR is within normal range. IR along with oxidative stress and inflammation also promote kidney disease. Recent evidence suggests that Angiotensin II and aldosterone may have significant metabolic effects and may contribute to the development and progression of insulin

resistant conditions. Some studies have reported Angiotensin II to induce insulin resistance via activation of Angiotensin II induced protein tyrosine phosphatase activation leading to dephosphorylation of the insulin receptor[18,19].

Greater degree of insulin resistance will predispose to renal injury by worsening renal hemodynamics through the elevation of glomerular filtration fraction and resultant glomerular hyperfiltration. IR along with oxidative stress and inflammation has been suggested to play a role in the development of albuminuria and declining kidney function. IR further predisposes to worsening of renal function by the deterioration of renal hemodynamics through mechanisms such as activation of sympathetic nervous system, sodium retention, decreased Na^+-K^+ATPase activity and increased GFR. Endoplasmic reticulum stress seems to be the factor linking inflammation and IR at the molecular level[20]. There is clear indication of prevelance of higher HOMA-IR in CKD case due to the onset of renal dysfunction associated with the decrease in glomerular filtration rate and there is disturbance in the insulin metabolism leading to hyperinsulinemia and post receptor defect resulting in insulin resistance. IR may be one of the mechanisms through which systemic inflammation may exert its deleterious effect on the cardio vasculature. Insulin mediated glucose uptake by the liver is normal in person with chronic renal failure, and tissue insensitivity to insulin is the primary cause of insulin resistance in patients with CKD. 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 Oberg et al[21].

The observation that IR in CKD is independent of impaired glycaemia and obesity signifies that greater surveillance is warranted, as IR is a modifiable risk factor. One of the limitations of the study is single estimation of the insulin resistance which may not represent average levels of this biomarkers overtime. For understanding the implication of insulin resistance in various stages of chronic kidney disease in better way, further studies including additional biomarkers can be employed in larger study population to elucidate their specific action in association to chronic kidney disease.

CONCLUSION

The present study shows presence of hyperinsulinemia and insulin resistance in patients with CKD. The cause of insulin resistance is multifactorial in CKD. Therapeutic intervention towards decreasing insulin resistance along with management of non diabetic CKD patients would be further beneficial in delaying the progression of renal dysfunction and towards reduction of cardiovascular mortality in chronic kidney disease cases.

AUTHOR'S CONTRIBUTIONS

Dr Lakshmi D, principal investigator and corresponding author of this article take the full responsibility for the scientific work performed by me. I, to the fullest extent, have been careful in the right selection of cases and controls by strict adherence to the inclusion and exclusion criteria. The study protocol has been performed with utmost care in regards to matters of ethical concern. I have acquired all the relevant data and analyzed it accordingly and agree to be accountable of all aspects of this work. The reagent kits (ELISA kits) procured by me from the distributor has helped me in bringing out the best possible results with respect to the measured parameters in my work. I ensure that the data reported in this research paper is representative of the original data. I also take the responsibility to ensure that the enquiries about the article will be promptly answered on behalf of my co-authors and that I would constantly appraise them on all the developments pertaining to this research work.

Dr. K. S. Meera, Professor & HOD, Department of Biochemistry, M. S. Ramaiah Medical College & Hospital, Bangalore and also my guide, has been instrumental in helping me conceptualize the research work with all her prized intellectual inputs and has helped me in the compilation and interpretation of data. She has been an outstanding mentor, motivator and has guided me in formatting and formulating the study design including its results, helped me in writing this manuscript. She has provided me the insight of the critical issues of the research work and the same has been incorporated in this study. She also suggested the journal IJCBR to publish our research work.

Dr. E. Mahesh - Consultant Nephrologist, M. S. Ramaiah Hospital, Bangalore, subject expert has played a major role during planning the study, recruitment of CKD cases, result analysis and meticulous direction in drafting of the publication. He has been very supportive and vocal about the outcome of the results and its significance during treatment of the diseased condition.

Conflicts of interest: None. All the financial expenses have been borne by the investigator.

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