

## ORIGINAL ARTICLE

# Effect of chronic kidney disease on serum resistin level

S Dan, P Aditya<sup>1</sup>, P Banerjee<sup>2</sup>, C Bal<sup>3</sup>, H Roy<sup>4</sup>, I Banerjee<sup>2</sup>

Departments of Biochemistry, <sup>2</sup>Pharmacology, <sup>3</sup>Physiology and <sup>4</sup>General Surgery, Calcutta Medical College, Kolkata, <sup>1</sup>Department of Life Sciences, Tejganj High School, Burdwan, West Bengal, India

## Abstract

**Background:** Chronic Kidney Diseases (CKD) of all etiologies are usually associated with Insulin Resistance (IR). Resistin is also a protein associated with IR. Some studies conducted abroad have shown that resistin level is higher among CKD patients.

**Objective:** To test if serum resistin level is significantly higher in CKD patients compared to normal individuals.

**Patients and Methods:** 96 CKD patients and 97 normal individuals were included in the study. Written informed consent was obtained from every individual.

**Results:** Serum resistin level was higher in CKD patients compared to control subjects. The difference in serum resistin level between two groups was statistically significant.

**Conclusion:** Our study is probably the first study in India comparing serum resistin levels of CKD patients vis-à-vis control subjects. Further cellular research may be needed to explore this relation.

**Key words:** Chronic kidney disease, HOMA-IR, insulin resistance, resistin

**Date of Acceptance:** 21-Mar-2014

## Introduction

Although great strides have been made in the field of medicine, chronic kidney disease (CKD) and end stage renal disease (ESRD) remain major public health challenges. Globally, the numbers of CKD patients and as a corollary, ESRD patients on maintenance hemodialysis have gone up in virtually all countries in last two decades.

In India, the approximate prevalence of CKD is 800 per million population (pmp), and the incidence of ESRD is 150-200 pmp.<sup>[1]</sup> In India, with a population base of 1 billion and an estimated incidence of ESRD of 100 pmp, approximately 100,000 patients develop ESRD each year.<sup>[2]</sup>

Chronic kidney disease of any etiology is associated with insulin resistance (IR) of primarily peripheral tissues resulting in varying degrees of hyperinsulinemia and glucose (Glu) intolerance.<sup>[3,4]</sup> Decreased response to insulin

is manifest already in mild renal dysfunction<sup>[5]</sup> and progresses with declining glomerular filtration rate (GFR).<sup>[3,6]</sup> The proposed link(s) between declining renal function and IR appear to act mainly in peripheral tissues,<sup>[3]</sup> but the exact mechanisms have so far not been clarified,<sup>[7]</sup> and a variety of possible pathological processes have been proposed.

Resistin is a protein secreted by monocytes, macrophages, bone marrow cells and adipose tissue.<sup>[8]</sup> It has been shown to inhibit the action of insulin *in-vitro*.<sup>[9-11]</sup> Its level is usually higher in patients with IR.

It has been proposed that resistin may be higher in CKD. A study has lent credence to this view.<sup>[12-14]</sup>

As the study linking resistin with CKD has been performed abroad, we intend to verify if the relationship of higher

### Access this article online

Quick Response Code:	Website: <a href="http://www.njcponline.com">www.njcponline.com</a>
	DOI: ***
	PMID: *****

### Address for correspondence:

Dr. Subhasish Dan,  
FE 367, Salt Lake, Kolkata - 700 106, West Bengal, India.  
E-mail: [subhasish.dan@gmail.com](mailto:subhasish.dan@gmail.com)

resistin with CKD holds well in Indian circumstances too.

## Materials and Methods

### Patients

Patients were recruited from the out-patient department of tertiary care hospital after obtaining ethical clearance. This study was performed in accordance with the Helsinki declaration and written informed consent was obtained from each participant. Ninety-six patients were included in CKD group, whereas 97 patients were included in the control group. The following selection criteria were used for patient recruitment:

### Inclusion criteria

Chronic kidney disease patients and body mass index (BMI), age and sex matched normal individuals.

### Exclusion criteria

We did not recruit obese patients, diabetics, metabolic syndrome patients, endocrinopathy patients and nephrotic syndrome patients for this study. For metabolic syndrome, diagnostic criteria by International Diabetes Federation were used.

As per the National Kidney Foundation Kidney Disease Outcome Quality Initiative guideline, patients with estimated GFR (eGFR)  $<60$  ml/min/1.73 m<sup>2</sup> for at least 3 months were included in CKD group. eGFR was calculated using Modification of Diet in Renal Disease formula.

None of the CKD patients was on hemodialysis or peritoneal dialysis.

### Study protocol

Before collection of data or blood sample, each patient was explained the details of the study including rationale, expected benefits, risk profile, confidentiality safeguards and study protocol. For some patients, help of appropriate interpreter(s) was taken. Only those patients who were willing to follow the study protocol and gave their written consent were included in this study. There was neither any financial cost nor any financial incentive for the patient for being part of the study.

Appropriate blood samples were collected from CKD patients. For estimation of serum insulin (Ins) level, serum high sensitive C-reactive protein (hsCRP) level, serum urea (UR) level, serum creatinine (Cr) level, fasting blood sample was drawn into serum (without anticoagulant) gel containing yellow color capped BD Vacutainer tubes. For plasma Glu level, blood sample were drawn into fluoride-oxalate anticoagulant containing gray color capped BD Vacutainer tubes. Both the afore-mentioned

samples were drawn on the same day (at closest possible intervals). All samples were immediately centrifuged, separated and stored at  $-70^{\circ}\text{C}$  until analysis for the relevant biochemical parameters. The following parameters were measured by Dimension RXL Max autoanalyzer:

1. Plasma Glu was measured by hexokinase principle
2. Plasma hsCRP was measured by turbidimetric principle
3. Plasma Ur was measured by urease principle
4. Plasma Cr was measured by alkaline picrate kinetic principle
5. Serum albumin was measured by bromocresol purple dye-binding method.

Serum resistin was measured using ELISA principle (LINCO Research, USA).

Serum Ins was measured by Abbott AxSYM is based on microparticle enzymes immunoassay technique.

Patients' recent-most blood/plasma/serum values of the afore-mentioned biochemical parameters were noted if already available, provided they were done on the same day.

Patients' primary etiology of nephropathy, disease history and treatment history were noted.

Patients' current medication details were noted.

Patients' demographic profiles including age, sex were noted.

Postdata collection, patients were divided into two groups- CKD and control. The serum levels of resistin, Ins, hsCRP, UR, Cr, albumin and plasma Glu between the two groups were compared for the presence or absence of statistical significance.

Homeostatic model assessment of IR<sup>[15]</sup> (HOMA-IR) was calculated by the formula:

$$\text{HOMA-IR} = \frac{\text{Fasting Serum Insulin } (\mu\text{IU} / \text{mL}) \times \text{Fasting Plasma Glucose } (\text{mg} / \text{dL})}{405}$$

### Statistical analysis

The statistical software Minitab 16 was used to analyze the data. Values of continuous variables were expressed as mean  $\pm$  one standard deviation unless otherwise indicated, and differences in mean values between two groups were analyzed using Student's *t*-test. Descriptive information regarding categorical variable were presented as frequency. Test for equality of two proportions was used for comparison of categorical data. All tests were two-tailed and considered statistically significant if  $P <$  level of significance, 0.05.

**Table 1: Comparison of anthropological, demographic, and biochemical statistics between CKD and control groups**

Parameters	CKD	Control	P value
Number	96	98	
Male/female	50/46	51/47	0.995
Age (years)	45.2 (9.1)	47.2 (8.7)	0.10
BMI (kg/m <sup>2</sup> )	27.6 (4.6)	26.8 (4.6)	0.23
Fasting plasma glucose (mg/dL)	101 (27)	97 (19)	0.236
Fasting serum insulin (mIU/L)	15.4 (2.5)	8.3 (1.6)	<0.001*
HOMA-IR	3.92 (0.15)	1.97 (0.14)	<0.001*
Resistin (ng/mL)	22.2 (3.4)	10.1 (1.9)	<0.001*
hsCRP (mg/L)	7.7 (4.9)	3.5 (1.6)	<0.001*
Creatinine (mg/dL)	2.9 (1.1)	0.9 (0.4)	<0.001*
Urea (mg/dL)	69 (18)	29 (9)	<0.001*
Serum albumin (g/dL)	3.9 (1.1)	4.4 (1.0)	0.001*

\*Denotes statistical significance. CKD=Chronic kidney disease; BMI=Body mass index; HOMA-IR=Homeostasis model assessment insulin resistance; hsCRP=High sensitive C-reactive protein

## Results

Serum fasting insulin ( $15.4 \pm 2.5$  vs.  $8.3 \pm 1.6$ ; mIU/mL), HOMA-IR ( $3.92 [0.15]$  vs.  $1.97 [0.14]$ ), serum hsCRP ( $7.7 \pm 4.9$  vs.  $3.5 \pm 1.6$ ; mg/L), serum UR ( $69 \pm 18$  vs.  $29 \pm 9$ ; mg/dL), serum Cr ( $2.9 \pm 1.1$  vs.  $0.9 \pm 0.4$ ; mg/dL), serum resistin ( $22.2 [3.4]$  vs.  $10.1 [1.9]$ ) levels were significantly higher in CKD patients compared to age and sex matched control individuals. Serum albumin level was significantly lower ( $3.9 \pm 1.1$  vs.  $4.4 \pm 1.0$ ; g/dL) in CKD patients compared with age and sex matched control individuals [Table 1].

## Discussion

Resistin is a 12.5-kDa cysteine-rich protein secreted mainly by adipocytes. Resistin circulates in serum in at least two distinct assembly forms that appear to have different levels of bioactivity.<sup>[16]</sup>

In animal studies, administration of recombinant resistin has been shown to impair Glu tolerance and decrease the action of insulin. The animals treated with resistin-neutralizing antibodies<sup>[17]</sup> show an increase in insulin sensitivity. Genetic studies in humans suggest that a polymorphism in the promoter region of the resistin gene can affect transcription factor binding,<sup>[18]</sup> thus influencing messenger ribonucleic acid (mRNA) levels<sup>[19]</sup> and may be associated with Glu intolerance in obese individuals.<sup>[20,21]</sup> Resistin has consequently been shown to be influenced by BMI.<sup>[22]</sup>

However, any clear consensus on the role of resistin in IR, hepatic and peripheral, is yet to emerge.<sup>[23,24]</sup> This lack of consensus may be due to the fact that mRNA level and protein expression of resistin may not always correlate.<sup>[25]</sup>

Our study confirms the finding of an earlier study<sup>[11]</sup> that plasma resistin level is higher in CKD patients in comparison to age and sex matched control (nonCKD) individuals. The HOMA-IR levels of two groups were also statistically significantly different.

Several factors may be responsible for elevation of resistin level in CKD.

As resistin is a 12.5 kDa protein, it is likely that this small protein will have similar renal clearance characteristics as  $\beta$ 2-microglobulin (13.7 kDa), which has been extensively studied.<sup>[26]</sup> If renal route, as is the case with  $\beta$ 2-microglobulin, is the principal excretory mechanism for resistin, then resistin level should rise with decline in GFR. This has been ascertained in an earlier study.<sup>[27]</sup>

Resistin production is mediated by pro-inflammatory cytokines.<sup>[28]</sup> Resistin also mediates production of inflammatory cytokines.<sup>[29-31]</sup> The principal sites of resistin production are adipose tissue, bone marrow<sup>[12]</sup> etc. The association between resistin level and levels of pro-inflammatory cytokines has already been proved.<sup>[32-35]</sup> CKD is a state of chronic inflammation, even at mild or moderate level of renal function impairment.<sup>[36,37]</sup> Resistin has been found to be elevated in nonobese CKD patients.<sup>[11]</sup> Antihypertensives viz. Losartan has been shown to ameliorate renal injury by controlling the adipocytokine imbalance.<sup>[38]</sup>

In cultured human vascular endothelial cells, resistin is able to induce expression of adhesion molecules vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, an effect that is inhibited by adiponectin.<sup>[20]</sup> *In vitro* studies have demonstrated resistin mRNA expression in peripheral blood monocytes, with increased levels after stimulation by interleukin (IL)-1, IL-6, and tumor necrosis factor- $\alpha$ , as well as with lipopolysaccharides.<sup>[19,21]</sup> It has also been demonstrated that plasma resistin levels correlate with markers of inflammation and predict coronary atherosclerosis in humans independently of CRP.<sup>[39]</sup>

## Conclusion

This study confirms the findings of earlier studies conducted abroad. To the best of our knowledge, ours is the first study exploring the association between CKD and serum resistin level in Eastern Indian population. Further confirmatory clinical studies and cellular research may be needed to explore this relation. Whether resistin may serve as an early marker of chronic inflammation and cardio vascular diseases in CKD patients may be explored in large scale multi centered studies.

## References

1. Agarwal SK, Srivastava RK. Chronic kidney disease in India: Challenges and solutions. *Nephron Clin Pract* 2009;111:c197-203.

2. Kher V. End-stage renal disease in developing countries. *Kidney Int* 2002;62:350-62.
3. Alvestrand A. Carbohydrate and insulin metabolism in renal failure. *Kidney Int Suppl* 1997;62:S48-52.
4. DeFronzo RA, Alvestrand A, Smith D, Hendler R, Hendler E, Wahren J. Insulin resistance in uremia. *J Clin Invest* 1981;67:563-8.
5. Kobayashi S, Maesato K, Moriya H, Ohtake T, Ikeda T. Insulin resistance in patients with chronic kidney disease. *Am J Kidney Dis* 2005;45:275-80.
6. Govaerts P. Physiopathology of glucose excretion by the human kidney. *BMJ* 1952;2:175-9.
7. Rigalleau V, Gin H. Carbohydrate metabolism in uraemia. *Curr Opin Clin Nutr Metab Care* 2005;8:463-9.
8. Fain JN, Cheema PS, Bahouth SW, Lloyd Hiler M. Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* 2003;300:674-8.
9. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, *et al.* The hormone resistin links obesity to diabetes. *Nature* 2001;409:307-12.
10. Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, *et al.* FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. *EMBO J* 2000;19:4046-55.
11. Kim KH, Lee K, Moon YS, Sul HS. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. *J Biol Chem* 2001;276:11252-6.
12. Axelsson J, Bergsten A, Qureshi AR, Heimbürger O, Bárány P, Lönnqvist F, *et al.* Elevated resistin levels in chronic kidney disease are associated with decreased glomerular filtration rate and inflammation, but not with insulin resistance. *Kidney Int* 2006;69:596-604.
13. Mills KT, Hamm LL, Alper AB, Miller C, Hudaihed A, Balamuthusamy S, *et al.* Circulating adipocytokines and chronic kidney disease. *PLoS One* 2013;8:e76902.
14. Marouga A, Dalamaga M, Kastania AN, Antonakos G, Thrasivoulides A, Kontelia G, *et al.* Correlates of serum resistin in elderly, non-diabetic patients with chronic kidney disease. *Clin Lab* 2013;59:1121-8.
15. 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;28:412-9.
16. Patel SD, Rajala MW, Rossetti L, Scherer PE, Shapiro L. Disulfide-dependent multimeric assembly of resistin family hormones. *Science* 2004;304:1154-8.
17. Muse ED, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, *et al.* Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest* 2004;114:232-9.
18. Cho YM, Youn BS, Chung SS, Kim KW, Lee HK, Yu KY, *et al.* Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia* 2004;47:559-65.
19. Smith SR, Bai F, Charbonneau C, Janderová L, Argyropoulos G. A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. *Diabetes* 2003;52:1611-8.
20. Ma X, Warram JH, Trischitta V, Doria A. Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. *J Clin Endocrinol Metab* 2002;87:4407-10.
21. Wang H, Chu WS, Hemphill C, Elbein SC. Human resistin gene: Molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. *J Clin Endocrinol Metab* 2002;87:2520-4.
22. Briffa JF, McAinch AJ, Poronnik P, Hryciw DH. Adipokines as a link between obesity and chronic kidney disease. *Am J Physiol Renal Physiol* 2013;305:F1629-36.
23. Asensio C, Cettour-Rose P, Theander-Carrillo C, Rohner-Jeanraud F, Muzzin P. Changes in glycemia by leptin administration or high-fat feeding in rodent models of obesity/type 2 diabetes suggest a link between resistin expression and control of glucose homeostasis. *Endocrinology* 2004;145:2206-13.
24. Maebuchi M, Machidori M, Urade R, Ogawa T, Moriyama T. Low resistin levels in adipose tissues and serum in high-fat fed mice and genetically obese mice: Development of an ELISA system for quantification of resistin. *Arch Biochem Biophys* 2003;416:164-70.
25. Rajala MW, Qi Y, Patel HR, Takahashi N, Banerjee R, Pajvani UB, *et al.* Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. *Diabetes* 2004;53:1671-9.
26. Karlsson FA, Groth T, Sege K, Wibell L, Peterson PA. Turnover in humans of beta 2-microglobulin: The constant chain of HLA-antigens. *Eur J Clin Invest* 1980;10:293-300.
27. Kielstein JT, Becker B, Graf S, Brabant G, Haller H, Fliser D. Increased resistin blood levels are not associated with insulin resistance in patients with renal disease. *Am J Kidney Dis* 2003;42:62-6.
28. Vendrell J, Broch M, Vilarrasa N, Molina A, Gómez JM, Gutiérrez C, *et al.* Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: Relationships in obesity. *Obes Res* 2004;12:962-71.
29. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, *et al.* Resistin promotes endothelial cell activation: Further evidence of adipokine-endothelial interaction. *Circulation* 2003;108:736-40.
30. Benomar Y, Gertler A, De Lacy P, Crépin D, Ould Hamouda H, Riffault L, *et al.* Central resistin overexposure induces insulin resistance through Toll-like receptor 4. *Diabetes* 2013;62:102-14.
31. Fu Y, Luo L, Luo N, Garvey WT. Proinflammatory cytokine production and insulin sensitivity regulated by overexpression of resistin in 3T3-L1 adipocytes. *Nutr Metab (Lond)* 2006;3:28.
32. Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR. Resistin messenger-RNA expression is increased by proinflammatory cytokines *in vitro*. *Biochem Biophys Res Commun* 2003;309:286-90.
33. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, *et al.* Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: A new insight into adipocytokine-endothelial cell interactions. *Biochem Biophys Res Commun* 2004;314:415-9.
34. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA. An inflammatory cascade leading to hyperresistinemia in humans. *PLoS Med* 2004;1:e45.
35. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, *et al.* Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003;300:472-6.
36. Pecoits-Filho R, Heimbürger O, Bárány P, Suliman M, Fehrman-Ekholm I, Lindholm B, *et al.* Associations between circulating inflammatory markers and residual renal function in CRF patients. *Am J Kidney Dis* 2003;41:1212-8.
37. Landray MJ, Wheeler DC, Lip GY, Newman DJ, Blann AD, McGlynn FJ, *et al.* Inflammation, endothelial dysfunction, and platelet activation in patients with chronic kidney disease: The chronic renal impairment in Birmingham (CRIB) study. *Am J Kidney Dis* 2004;43:244-53.
38. Jian DY, Chao YW, Ting CH, Huang SW, Chang CF, Juan CC, *et al.* Losartan ameliorates renal injury, hypertension, and adipocytokine imbalance in 5/6 nephrectomized rats. *Eur J Pharmacol* 2013;709:85-92.
39. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* 2005;111:932-9.

**How to cite this article:** ???

**Source of Support:** Nil, **Conflict of Interest:** None declared.