IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 6 Ver. II (Nov. – Dec. 2017), PP 05-10
www.iosrjournals.org

Circulating Resistin Concentrations, Inflammatory Biomarkers and Insulin Resistance in Iraqi Patients with Chronic Kidney Failure

Ass.Prof Dr. Manal kamal Rasheed[1], Ass. Prof, Dr.Maysaa Jalal Majeed[2], Lecturer Dr. Wafa Mansor Merza[3], Dr. Mohammed kamal Rasheed[3], Prof Dr. Halla Ghazi Mahmoud[4]

[1](Ph.D.clinical biochemistry .medicine college/Baghdad university manalri@yahoo,com/009647901739372
[2](PhD . clinical biochemistry .medicine college/Baghdad university) mysjalal@yahoo.com/009647701658660
[3](PhD.clinical biochemistry .dentistry college/Baghdad university
wawamama528@yahoo.com/009647701831368.
[3]AL-Karama Hospital (F.IC.M.S(Consultant urologist) www.dr.mohammedkamal63@gmail.com
[4](PhD.clinical biochemistry .medicine college/Baghdad university) Halla_ghazi@yahoo.com/009647901724851

Abstract:
Background: *Resistin in humans is mainly produced by blood-derived leukocytes and mononuclear cells. Resistin antagonizes the effects of insulin on glucose metabolism in liver and skeletal muscle, interacts with and reinforces inflammatory pathways.*
Objective: *Evaluation of the resistin level as inflammatory factor in patients with chronic renal failure.*
Patients and Methods: *Study was conducted at the department of Biochemistry, College of Medicine / University of Baghdad and AL-Karama Hospital during the period from March 2016 to March 2017.*
This cross-sectional study was composed of 50 consecutive patients with CRF according to the National Kidney Foundation practice guidelines and 30 apparently healthy control subjects was matched. Measurements of fasting plasma glucose and serum levels of triglyceride, total cholesterol, creatinine were done by colorimetric method, followed by calculation of estimated glomerular filtration rate (eGFR). Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) index. Serum levels of resistin, high sensitivity CRP were estimated by ELISA technique. Excluding criteria include diabetic obese subjects.
Results: *The levels of resistin, insulin , CRP and HOMA were significantly higher in patients with CKD than in the control subjects ($p \leq 0.05$). Patients with elevated CRP (>6 mg/L) had significantly higher resistin levels than those with lowered CRP (≤6 mg/L). A Significant positive correlation was demonstrated between serum levels of resistin and serum levels of insulin, HOMA and creatinine ($r=0.54, r=0.77, r=0.302$, respectively, $p \leq 0.05$) and a significant negative correlation was found between serum resistin and eGFR ($r=-0.2$, $p \leq 0.05$).*
Conclusion: *The result of this study revealed that high level of resistin in patients with high sensetive C – reactive protein may suggested that resistin elevation related to inflammation associated with chronic renal failure.*
Key words*: chronic kidney disease, resistin, insulin resistance, Inflammation.*

Date of Submission: 25-10-2017

I. Introduction

Resistin is a recently discovered cysteine-rich plasma protein that belongs to a family of polypeptides called resistin-like molecules. Although classified as an adipokine, resistin in humans is mainly produced by blood-derived leukocytes and mononuclear cells, both within and outside the adipose tissue[1,2]. The physiological role and pathophysiologic importance of resistin in humans are unclear. Resistin antagonizes the effects of insulin on glucose metabolism in liver and skeletal muscle, interacts with and reinforces inflammatory pathways, and may promote endothelial cell activation. Increased resistin levels have been associated with obesity, insulin resistance, metabolic syndrome, type 2 diabetes, and increased cardiovascular risk, although the evidence is not consistent[3].

There is evidence that resistin has proinflammatory properties and it is abundant in inflammatory diseases as resistin is a protein with a relatively low molecular weight, it is assumed that reduced renal excretory function exerts an influence on its concentration[4]. Plasma resistin level has recently been shown to be associated with markers of CKD, and it is speculated that inflammatory, metabolic, and vascular abnormalities associated with increased circulating resistin levels may have a pathogenic role in CKD. In addition, resistin can modulate several molecular pathways involved in metabolic, inflammatory, and autoimmune diseases[5].

Chronic kidney disease (CKD) is a major public health problem. It is 3 □4 times more common in Africa than in developed countries, and the reported prevalence of chronic renal failure in Egypt is 225 per million. Adipose tissue is no longer considered to be an inert tissue for storing fat, but is known to actively secrete a number of adipokines and cytokines that are involved in the regulation of various metabolic processes[6]. In patients with uremia, adipose tissue is an important source of molecules responsible for the metabolic disturbances seen in these patients. Some of these molecules act as pro-inflammatory agents, contributing to the maintenance and enhancement of the chronic inflammatory response. There is evidence that these molecules may have multiple effects, including modulating insulin signaling and impairing endothelial health and vascular outcome [7].

Insulin resistance is the central pathophysiological process of the metabolic syndrome, a well-established and major risk factor for the development of cardiovascular disease[8].Chronic kidney disease (CKD) of any etiology is associated

with insulin resistance of primarily peripheral tissues resulting in varying degrees of hyperinsulinemia and glucose intolerance. Decreased response to insulin is manifest already in mild renal dysfunction and progresses with declining glomerular filtration rate (GFR)[4], The proposed link(s) between declining renal function and insulin resistance appear to act mainly in peripheral tissues, but the exact mechanisms have so far not been clarified[6], and a variety of possible etiologies are proposed.

Patients and Methods:

Study was conducted at the department of Biochemistry, College of Medicine, University of Baghdad during the period from March 2016 to March 2017. Ethical approval was received from the scientific Committee of Biochemistry department/ College of Medicine/ University of Baghdad and AL-Karama Hospital.

This study included 80 subjects who attended AL-Karama hospital, in the year 2017. The study group consisted of 50 patients (eGFR <60 mL/min/1.73 m^2) (Group I), with age ranging between(46 – 70) years (26 males and 24 females), and 30 apparently healthy individuals(Group ll), with normal kidney function, not receiving medication for any condition, and with no history of diabetes mellitus (DM).Age and sex of both groups (l & ll) was matched. Obese individuals and subjects with DM were excluded from this study to avoid obesity and DM as known causes of insulin resistance. Thorough assessment, including history and physical examination and blood pressure (BP) measurement was carried out. The weight and height of the subjects were used to calculate the body mass index (BMI) using the following formula[9]:
Weight (kg)/height $(m)^2$. Overweight was defined as BMI ≥25 and<30 kg/m2, obesity defined as BMI >30 kg/m^2.

The levels of fasting blood glucose, serum levels of triglycerides,serum total cholesterol, urea and creatinine were estimated using a Hitachi 912 chemistry analyzer (Roche). Calculation of eGFR was made using the abbreviated modification of diet in renal disease (MDRD) equation[10]. Human insulin was estimated by enzyme linked immunosorbent assay technique using kits supplied by BioSource. Fasting plasma glucose and fasting insulin levels were used to calculate the homeostasis model assessment (HOMA)[11]. CRP level in serum was estimated using high sensitivity CRP ELISA kit by GenWay[12]. Serum resistin was estimated by enzyme linked immunosorbent assay technique using kits supplied by BioVendor Laboratorni Medicina[13].

Blood pressure for any individual participant was calculated as the average of all available systolic and diastolic readings. Hypertension was defined as the presence of a mean systolic BP≥ 140mmHg and /or diastolic BP≥90 mmHg and /or use of antihypertensive medication.
The patients were subdivided into two groups according toblood CRP level (>6 mg/L & ≤ 6 mg/L).

Statistical analyses:

Data are presented as mean ±S.D. Differences between two groups were analyzed by the unpaired Student's *t*-test, A *P* value of ≤0.05 was considered statistically significant. Correlation coefficient used to find the correlation between studied markers by using Pearson correlation. SPSS version 17 was used in assessment of the study's data.

II. Results

Patients with renal failure were found to have significantly higher systolic and diastolic BP than the healthy control subjects (table 1). The mean and interquartile range of systolic BP were 135.0 and 120.0 mmHg, respectively (p≤0.05) and the corresponding diastolic BP figures were 90.0 and 80.0 mmHg, respectively (p≤0.05).

Table 1 shows the biochemical data in the study patients . The (mean± SD) level of serum total cholesterol was significantly higher in the patients with renal failure (208.643±6.8)mg/dl than in the control subjects (185.1∓ 29.4) mg/dl, p≤0.05. The (mean± SD) level of serum triglyceride was significantly higher in the patients with renal failure (111.4±15.6)mg/dl than in the control subjects(107.5±18.8) mg/dl ,p≤0.05. The (mean± SD) level of serum creatinine was significantly higher in the patients with renal failure, (573.4±260.5) mmol/l than in the control subjects (85±17.68) mmol/l (p≤0.05), The mean± SD level of serum urea was significantly higher in the patients with renal failure,(25.1±10.9) mmol/l than in the control subjects, (4.2±1.1) mmol/l (p≤0.05) and their eGFR was significantly lower, (9.9±1.6) mL/min/1.73 m^2, than that of the control subjects, (195.71± 68.15)mL/min/1.73 m^2 (p≤0.005). The (mean ±SD) fasting blood insulin level was significantly higher (13.3∓12.19)µU/mL in patients than in the control subjects (7.5∓4.7) µU/mL (p≤0.005) and the (mean ±SD) of HOMA level was significantly higher in the patients(9.05±7.9) compared with the control subjects (1.6±1.1) ,(p≤0.005). The (mean ±SD) of CRP level was significantly higher in the patients with renal failure (25.1±10.9) mmol/l than in the control subject (0.66±0.44) mg/dl, (p≤0.005). The (mean ±SD) of serum resistin level was significantly higher in patients with renal failure (25.13±7.07) ng/mL than in the control subjects (12.86±2.34) ng/mL (p≤0.005).

Table 1. Clinical characteristics of patients with chronic kidney failure and control subjects.

Variable	CRF patients n=50	Control n=30	P -value
Systolic Bp (mmHg)	135	120	P≤0.05
Diastolic BP (mmHg)	90.0	80.0	P ≤0.05
Fasting blood glucose (mmol/l)	4.8±1.0	4.9±0.7	P>0.05
Blood urea (mmol/L)	25.11±0.9	4.21±.1	P≤0.005
Creatinine (mmol/l)	573.42±60.5	85±17.68	P≤0.005
HbA1c%	5.18 ±0.27	5.08 ±0.25	P>0.05
Serum Insulin(µu/ml)	13.3±12.19	7.5±4.7	P≤0.005
HOMA-Insulin resistance	9.0±57.9	1.6±1.1	P≤0.005
Serum cholesterol (mg/dl)	208.64±36.8	185. ±129.4	P≤0.05
Serum triglycerides (mg/dl)	111.4±15.6	107.5±18.8	P≤0.05
eGFR (mL/min/1.73 m2)	9.9±1.6	195.71± 68.15	P≤0.005
Resisti (ng/mL)	25.13±7.07	12.86±2.34	P≤0.005
C-reactive protein (CRP) (mg/L)	3.37± 3.1	0.66±0.44	P≤0.005

Data are presented as mean± SD for creatinine , urea, FBG ,resistin, triglycerides, total cholesterol, eGFR, insulin, HOMA and CRP .Statistically significant (p≤0.05)
eGFR: Estimated glomerular filtration rate, HOMA: Homeostasis model assessment

There was significant positive correlation between serum levels of resistin and insulin (r=0.54, p≤0.05), resistin and HOMA (r=0.77, p≤0.05), and resistin and creatinine (r=0.302, p≤0.05). A significant negative correlation was found between resistin and eGFR (r=-0.2, p≤0.05), as shown in table 2. With regard to analysis of the correlations between the various different parameters, a significant negative correlations were seen between eGFR and resistin (r=- 0.2 ,p≤0.05 fig. 1), insulin (r=-0.23,p=0.05,fig. 2) and HOMA (r=-0.24, p≤0.05,fig. 3) while resistin was correlated positively with insulin (r=0. 54 ,p≤0.005,fig. 4).

Table (2): Correlation coefficients among eGFR, resistin, insulin, HOMA, creatinine, total cholesterol and triglycerides in patients with chronic kidney disease (CKD) (n=50).

Parameters	e GFR	Resistin	Insulin	HOMA
S.Resistin p-value	r=-0.2* 0.05			
S.Insulin p-value	r=-0.26* 0.05	r= 0.54* 0.05		
HOMA P-value	r=-0.26* 0.05	r= 0.77* 0.05	r=0.97* 0.0005	
S.creatinine p-value	r=-0.86* 0.0005	r=0.30* 0.019	r=0.3* 0.021	r=0.24 0.062
S.T-cholesterol p-value	r=-0.02 0.907	r=0.01 0.926	r=0.05 0.718	r=0.04 0.774
S.triglyceride p-value	r=-0.22 0.094	r=0.14 0.291	r=0.22 0.099	r=0.19 0.146

* Statistically significant (p0≤.05)

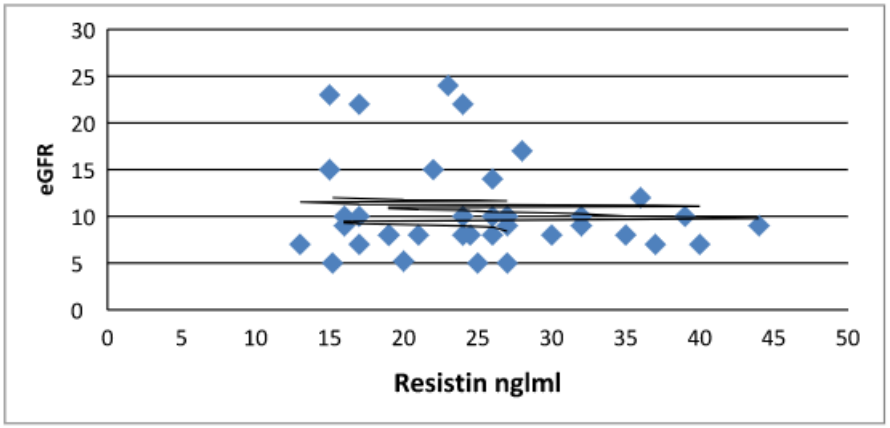

Figure 1: Correlation of glomerular filtration rate (eGFR) with serum resistin in patients with chronic renal failure (p≤0.05, r=-0.2).

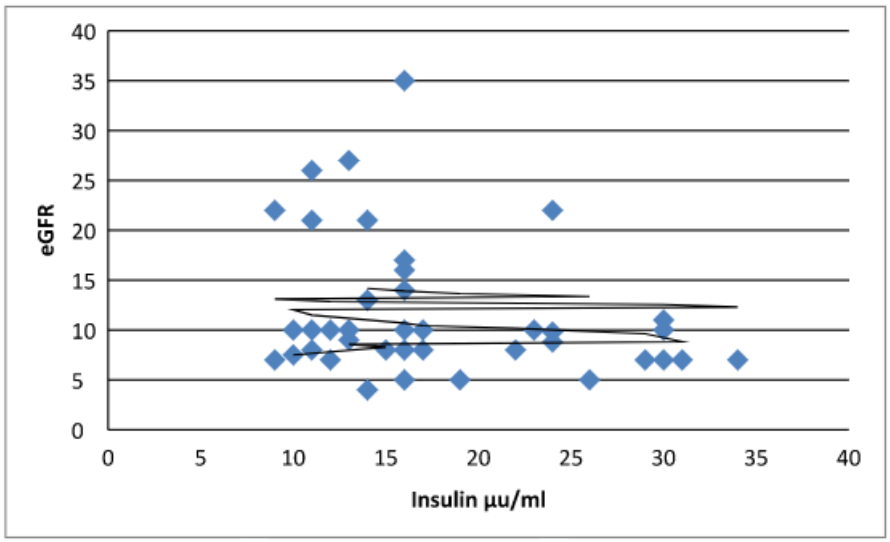

Figure 2: Correlation of glomerular filtration rate (eGFR) with serum insulin in patients with chronic renal failure (p=0.05; r=-0.26)

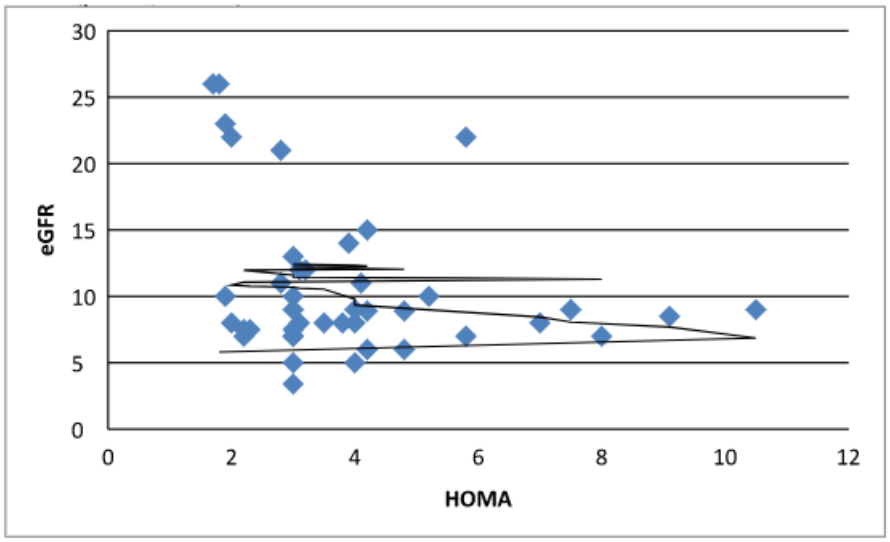

Figure 3: Correlation of estimated glomerular filtration rate (eGFR) with homeostasis model assessment (HOMA) in patients with chronic renal failure (p≤0.05,r= -0.24).

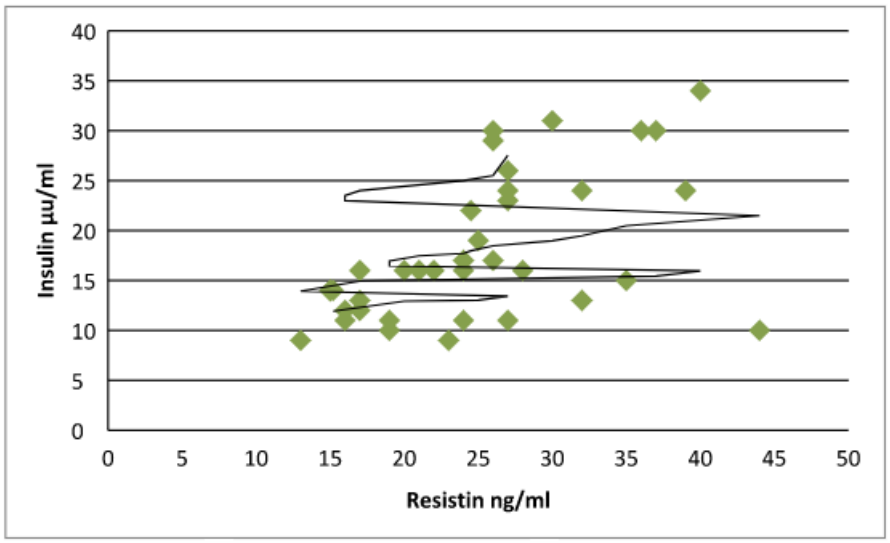

Figure 4: Correlation of resistin with insulin in patients with chronic renal failure (p≤0.005, r=0.54).

Table 3 shows the (mean± SD) level of serum resistin was significantly higher (28± 9.4) ng/ml in the patients with CRP >6 mg/dl, than in patient with CRP≤6 was (20.5+-6,25) ng/ml.p≤0.05.

Table3: Mean ±SD of serum resistin in patients with chronic renal failure grouping according to CRP level.

Variable	CRP> 6 mg/dl	CRP ≤ 6 mg/dl	p-value
S .resistin (ng/ml)	28±9.4	20.5±6.25	P≤0.05

Chronic renal failure Patients with CRP >6 had significantly higher level of resistin than chronic renal failure Patients with CRP ≤6,these finding may help to present the role of resistin as inflammatory factor in patients with chronic renal failure.

III. Discussion

The study findings in agreement with the previous researches, they reported that circulating serum resistin levels like other adipokines such as leptin and adiponectin were markedly elevated in patients with renal function impairment[14]. Several factors might contribute to elevated circulating resistin levels in chronic kidney disease (CKD), suggestion of the possible cause that increased levels of resistin due to decreased GFR and increased inflammatory activity in CKD may be one factor behind the insulin resistance syndrome present in these patients [8]. Park and Lindholm [15] recorded that uremic toxins may be cause an acquired defect in the insulin-receptor signaling pathway, and increased inflammation in uremia further develop insulin resistance. the study was in harmony with previous studies, a high significant difference was detected in the serum level of resistin between patients with CRF and control subjects, A significant positive correlation between resistin and creatinine, a negative correlation between resistin and eGFR were shown, suggesting that resistin concentration depends on renal function and is correlated with the severity of the renal disease. In agreement with these findings, the papers of Kielstein et al, [16] Diez et al, [17] and Ziegelmeier et al [18] they reported that various adipokines, including resistin,

were significantly elevated in patients with CKD compared with control subjects and the regulation of these adipokines in vivo is strongly dependent on renal function.

Nusken et al,[19] suggested that renal function is an important factor in the regulation of the systemic levels of resistin. Risch et al,[20] failed, however, to foundan association between GFR and serum resistin at GFR >60 mL/min/1.73 m^2, suggesting that resistin level in mildly impaired and normal renal function is influenced by factors other than GFR.

Al-Harithy and Al-Ghamdi [21] found that resistin was correlated with insulin and HOMA in lean, overweight and obese non-diabetic and diabetic subjects. Yaturu et al,[22] also found this correlation in CKD and considered that resistin represents a novel link among metabolic signals, inflammation, and atherosclerosis in CKD.

Anderson et al,[23]and Menzaghi C,[24] reported that resistin function as an inflammatory endocrine or paracrine signal antagonistic to insulin activity and contributory to metabolic and atherogenic changes in human inflammation in patients with CKD. Conversely, several other researchers found no association of resistin with insulin resistance. Kielstein et al,[16] stated that a greater than 5-fold increase in resistin blood levels was not associated with tumble in insulin sensitivity in patients with renal disease.

Among the studies dispute resistin and GRF, Dimitriadis K [25] Axelsson et al,[26] were documented that raised resistin levels in CKD were associated with decreased GFR and inflammation, but not with insulin resistance. These conflicting data may reflect variations in the design of the studies and lack of adjustment for potential confounding factors. It is also possible that resistin is a marker for, or contributes to insulin resistance only in specific populations[27].

However no correlation was found between serum levels of resistin and total cholesterol in patients with CRF in the study, these findings are consistent with those of Kielstein et al[16] and Yaturu et al.[22]In contrast, Taskapan et al,[28] and Park and Lindholm[15]showed that serum resistin level was positively correlated with triglycerides in patients on continuous peritoneal dialysis. This discrepancy could be explained by the different treatment applied to patients of the first and higher BMI in the patients of the latter study.

IV. Conclusion

Findings of high level of resistin in patients with high sensetive C - reactive protein may suggested that resistin elevation related to inflammation associated with chronic renal failure

References:

[1]. The *American Journal of Kidney Diseases (AJKD)*, the official journal of the National Kidney **May 2017**Volume 69, Issue 5, p555-712, e21-e24

[2]. McTernan PG, Kusminski CM, Kumar S. Resistin. Curr Opin Lipidol. 2006;17:170 –175

[3]. Menzaghi C, Xu M, Salvemini L, De Bonis C, Palladino G, Huang T, et al. Circulating adiponectin and cardiovascular mortality in patients with type 2 diabetes mellitus: evidence of sexual dimorphism. Cardiovasc Diabetol. 2014;13: 130 "In press".

[4]. Lorena Ortega Moreno1, Lucia Salvemini1, Christine Mendonca2, Massimiliano Copetti3,et al: Serum Resistin and Glomerular Filtration Rate in Patients with Type 2 Diabetes: | DOI:10.1371/journal.pone.0119529 March 26, 2015

[5]. Kawamura R, Doi Y, Osawa H, Ninomiya T, Hata J, Yonemoto K, et al. Circulating resistin is increased with decreasing renal function in a general Japanese population: the Hisayama Study. Nephrol DialTransplant. 2010; 25: 3236–3240. doi: 10.1093/ndt/gfq155 PMID: 20339098

[6]. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int. 2011; 80: 17–28. doi: 10.1038/ki.2010.483 PMID: 21150873

[7]. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality.A collaborative meta-analysis of high-risk population cohorts. Kidney Int. 2011; 79: 1341–1352. doi: 10.1038/ki.2010.536 PMID: 21307840

[8]. De Cosmo S, Menzaghi C, Prudente S, Trischitta V. Role of insulin resistance in kidney dysfunction: insights into the mechanism and epidemiological evidence. Nephrol Dial Transplant. 2013; 28: 29–36.doi: 10.1093/ndt/gfs290 PMID: 23048172

[9]. Babar, Sultan (March 2015). "Evaluating the Performance of 4 Indices in Determining Adiposity". Clinical Journal of Sports Medicine. Lippincott Williams & Wilkins) Retrieved August 2015,2: 183.

[10]. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150: 604–612. PMID: 19414839

[11]. Matthews DR, Hosker JP, Rudnski AS, Naylor BA, et al. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985, 28:412–419

[12]. Yukin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? ArterioscIerThromb Vasc Biol 1999, 19:972−978

[13]. Sheng CH, DI J, J in Y, Zhang YC, et al.: Resistin is expressed in human hepatocytes and induces insulin resistance. Endocrine 2008

[14]. Stenvinkel P, Marchlewska A,Pecoits-Filho R, HeimbürgerO, Zhang Z, Hoff C, Holmes C, Axelsson J, Arvidsson S,Schalling M, Barany P,Lindholm B and Nordfors L): Adiponectin in renal disease: relationship to phenotype and genetic variation in the gene encoding adiponectin. Kidney Int ; (2004)65: 274–281.

[15]. Pak SH, Lindhol M B. Definition of metabolic syndrome in peritoneal dialysis. Perit Dial Int 2009, 29(Suppl 2):137–144

[16]. Kielstein JT, Becker B, G raf S, Brabant G, etal:.Increased resistin blood levels are not associated with insulinresistance in patients with renal disease. Am J Kidney Dis2003, 42:62–66

[17]. Diez JJ, Iglesuas P, Frnande Z & Reyes MJ, et al:. Serum concentrations of leptin, adiponectin and resistin, and their relationship with cardiovascular disease in patients with end-stage renal disease. Clin Endocrinol(Oxf) 2005, 62:242–249

[18]. Ziegelmeier M, B achmann A, Seeger J, Lossner Uetal: Adipokines influencing metabolic and cardiovascular disease are differentially regulated in maintenance hemodialysis. Metabolism 2008, 57:1414−1421

[19]. Nusken KD, Kratzsch J, Wienholz V, Stohr W, etal:. Circulating resistin concentrations in children depend on renalfunction. Nephrol Dial Transplant 2006, 21:107–112

[20]. Risch L, Saely C, Hoefle G, Rein P, etal:.Relationship between glomerular filtration rate and the adipokinesadiponectin, resistin and leptin in coronary patients with predominantly normal or mildly impaired renal function.Clin Chim Acta 2007, 376:108–113

[21]. AL&Harithy RN, AL&Ghamdi : Serum resistin, adiposity and insulin resistance in Saudi women with type 2 diabetes mellitus. Ann Saudi Med 2005, 25:283−287
[22]. Yaturu S, Reddy RD, Rains J, Jain SK : Plasma and urine levels of resistin and adiponectin in chronic kidney disease. Cytokine2007, 37:1−5
[23]. Anderson PD, Meta NN, Wolfe ML, Hikle CC, etal:. Innate immunity modulates adipokines inhumans. J Clin Endocrinol Metab 2007, 92:2272−2279
[24]. Menzaghi C, Salvemini L, Fini G, Thompson R, Mangiacotti D, Di Paola R, et al. Serum resistin and kidney function: a family-based study in non-diabetic, untreated individuals. PLoS One. 2012; 7: e38414.doi: 10.1371/journal.pone.0038414 PMID: 22701635
[25]. Dimitriadis K, Tsioufis C, Selima M, Tsiachris D, Miliou A, Kasiakogias A, et al. Independent association of circulating resistin with glomerular filtration rate in the early stages of essential hypertension. J Hum Hypertens. 2009; 23: 668–673. doi: 10.1038/jhh.2009.12 PMID: 19262583
[26]. Axlsson J, Bergsten A, Qureshi AR, Heimburger O, et al.: Elevated resistin levels in chronic kidneydisease are associated with decreased glomerular filtration rate and inflammation, but not with insulin resistance.Kidney Int 2006, 69:596–604
[27]. De Cosmo S, Copetti M, Lamacchia O, Fontana A, Massa M, Morini E, et al. Development and validation of a predicting model of all-cause mortality in patients with type 2 diabetes. Diabetes Care. 2013;36: 2830–2835. doi: 10.2337/dc12-1906 PMID: 23637348
[28]. Taskapan MC, Taskapan H, Sahin I, Keskin N L, etal. :Serum leptin, resistin, and lipid levels in patients withend stage renal failure with regard to dialysis modality. RenFail 2007, 29:147−154

Ass.Prof Dr. Manal kamal Rasheed. "Circulating Resistin Concentrations, Inflammatory Biomarkers and Insulin Resistance in Iraqi Patients with Chronic Kidney Failure." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 12.6 (2017): 05-10