Estimation of resistin in chronic kidney disease

OBJECTIVE To compare the serum level of resistin in patients with chronic kidney disease (CKD) with that of normal control subjects and to elucidate the association of resistin with insulin resistance and markers of inflammation in CKD. METHOD Study was made of 60 patients with CKD according to the National Kidney Foundation practice guidelines and 20 apparently healthy control subjects in the age range, 46−70 years. Obese individuals and those with diabetes mellitus were excluded. Measurements were made of fasting plasma glucose and serum levels of triglycerides, total cholesterol, creatinine, C-reactive protein (CRP), resistin and insulin, followed by calculation of estimated glomerular filtration rate (eGFR) and homeostasis model assessment (HOMA). RESULTS The levels of resistin, insulin and CRP and HOMA were all higher in patients with CKD than in the control subjects (p<0.05). The serum level of resistin was higher in patients with stage 5 than in those with stage 4 CKD (p<0.05). Patients with elevated CRP (>6 mg/L) had statistically significantly higher resistin levels than those with lower CRP (≤6 mg/L). Positive correlation was demonstrated between serum level of resistin and the levels of insulin and creatinine (r=0.856, r=0.302, respectively, p<0.05) and negative correlation between serum resistin and eGFR (r=-0.285, p<0.05). CONCLUSIONS Resistin was found to be associated with CKD and its blood level increased with progressive impairment of renal function. Resistin was correlated with CRP in CKD and this may be indicative of a link with metabolic and cardiovascular complications.

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.¹ 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.² 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.³

Resistin is a 12.5 kD cysteine-rich plasma protein that belongs to a family of polypeptides called resistin-like molecules.⁴ Although it is classified as an adipokine, resistin in humans is produced mainly by blood-derived leukocytes and mononuclear cells, both within and outside the adipose tissue.¹ There is evidence that resistin has proinflammatory properties and it is abundant in inflammatory diseases.⁵ 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.⁷ 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.⁶ In addition, resistin can modulate several molecular pathways involved in metabolic, inflammatory, and autoimmune diseases.⁸

The aim of this study was to compare the serum level of resistin in patients with CKD and matched control subjects, and to investigate the possible association of serum resistin level with estimated glomerular filtration rate (eGFR), insulin resistance and the inflammatory marker C-reactive protein (CRP) in patients with CKD.
MATERIAL AND METHOD

This study included 80 subjects who attended Kasr ElEiny Hospital, Cairo University in the year 2009. The study protocol was approved by the medical research ethics committee of the hospital and written informed consent was secured from each subject. The study group consisted of 60 patients diagnosed with CKD according to the National Kidney Foundation practice guidelines (eGFR <60 mL/min/1.73 m³) (Group I), with age ranging between 46 and 70 years (36 males and 24 females), and 20 apparently healthy individuals, with normal kidney function, not receiving medication for any condition, and with no history of diabetes mellitus (DM), matched for age and sex, as the control group (Group II). 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: Weight (kg)/height (m)². Overweight was defined as BMI ≥25 and <30 kg/m² and obesity BMI ≥30 kg/m².10

The patients with CKD (Group I) were then sub-grouped according to their eGFR into Group Ia: 47 cases with eGFR <15 mL/min/1.73 m³ (stage 5), and Group Ib: 13 cases with eGFR ≥15–29 mL/min/1.73 m³ (stage 4). The staging is that of the National Kidney Foundation practice guidelines for CKD.9 Investigations for the study included: (a) Abdomino-pelvic ultrasonography to evaluate kidney damage and associated complications, and (b) laboratory tests for which venous blood samples were obtained after an overnight fast of >10 hours. The levels of fasting plasma glucose and serum total cholesterol, triglycerides 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.11 Human insulin was estimated by enzyme linked immunosorbent assay technique using kits supplied by BioSource.11 Fasting plasma glucose and fasting insulin levels were used to calculate the homeostasis model assessment (HOMA).12

CRP level in serum was estimated using high sensitivity CRP ELISA kit by GenWay.13 Serum resistin was estimated by enzyme linked immunosorbent assay technique using kits supplied by BioVendor Laboratorni Medicina.14

Statistical methods

All variables were tested with the Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Normally distributed variables were expressed as mean ± standard deviation (SD). For comparison of means, the Student’s t-test was used. Non-normally distributed variables were reported as median (interquartile range), and the nonparametric Mann-Whitney test was used for comparison of medians. Relationships between parameters were evaluated by Pearson correlation when the variables were normally distributed; otherwise, Spearman correlation was used. A p-value of less than 0.05 was considered statistically significant. Analysis of the data was made using Statistical Package for Social Sciences (SPSS) v.15 (SPSS Incorporation, Chicago, Illinois).

RESULTS

Patients with CKD were found to have significantly higher systolic and diastolic BP than the healthy control subjects (tab. 1). The median and interquartile range of systolic BP were 140.0 (122.5–160.0) and 120.0 (112.5–138.7) mmHg, respectively (p<0.05) and the corresponding diastolic BP figures were 90.0 (80.0–90.0) and 80.0 (70.0–87.5) mmHg, respectively (p<0.05).

Table 2 shows the biochemical data in the study patients. The mean level of serum creatinine was higher in the patients with CKD, 5.83±1.95 mg/dL, than in the control subjects, 0.538±0.29 mg/dL (p<0.05), and their median eGFR was found to be lower, 9.65 (7.85–12.83) mL/min/1.73 m², than that of the control subjects, 215.10 (132.77–331.77) mL/min/1.73 m² (p<0.05). The median fasting blood insulin level was 16.50 (12.77–23.15) µU/mL in patients and 9.90 (8.40–12.18) µU/mL in the control subjects (p<0.0005) and the median HOMA was 3.90 (3.00–4.80) in the patients compared with 2.10 (1.70–2.75) in the control subjects (p<0.05). The difference in the median of CRP between the two groups was also significant (p<0.05). The mean serum resistin level was higher in patients with CKD, 25.13±7.07 ng/mL, than in the control subjects, 12.86±2.34 ng/mL (p<0.05).

Comparing resistin in the two stages of CKD, the serum level of resistin was found to be significantly higher in Group Ia (stage 5 CKD) than Group Ib (stage 4 CKD), 26.42±6.86 vs 20.44±5.90 ng/mL (p<0.05), as shown in table 3.

When the patients with CKD were sub-grouped according to their serum levels of CRP into Group A patients with CRP ≤6 and Group B with CRP >6 ml/L, the mean

Table 1. Clinical characteristics of patients with chronic kidney disease (CKD) and control subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CKD patients (n=60)</th>
<th>Control subjects (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.80 (21.57–26.80)</td>
<td>23.80 (21.90–24.85)</td>
<td>0.56</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>140.0 (122.5–160.0)</td>
<td>120.0 (112.5–138.7)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>90.0 (80.0–90.0)</td>
<td>80.0 (70.0–87.5)</td>
<td>0.022*</td>
</tr>
</tbody>
</table>

Data are presented as median (25th–75th percentiles)

*Statistically significant (p<0.05)

BMI: Body mass index, BP: Arterial blood pressure
serum resistin level was found to be higher in Group B (27.03±5.76 ng/mL) than in Group A (20.6±8.27 ng/mL) (p<0.05), as shown in table 4.

Significant positive correlation was demonstrated between serum levels of resistin and insulin (r=0.856, p<0.05), resistin and HOMA (r=0.801, p<0.05), and resistin and creatinine (r=0.302, p<0.05). Significant negative correlation was found between resistin and eGFR (r=-0.285, p<0.05), as shown in table 5.

With regard to analysis of the correlations between the various different parameters, significant negative correlations were seen between eGFR and resistin (p=0.027; r=-0.285) (fig. 1), insulin (p=0.016; r=-0.310) (fig. 2) and HOMA (p=0.015; r=-0.313) (fig. 3) while resistin was correlated positively with insulin (p<0.0005, r=0.856) (fig. 4).

**Table 2.** Biochemical data in patients with chronic kidney disease (CKD) and control subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CKD patients (n=60)</th>
<th>Control subjects (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (TGs) (mg/dL)</td>
<td>121.5 (79.0–175.7)</td>
<td>95.0 (72.5–111.5)</td>
<td>0.071</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>178.0 (130.0–223.0)</td>
<td>195.0 (165.0–219.7)</td>
<td>0.166</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>5.83±1.95</td>
<td>0.54±0.29</td>
<td>0.0005*</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>9.65 (7.85–12.83)</td>
<td>215.10 (132.77–331.77)</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Fasting plasma glucose (FPG) (mg/dL)</td>
<td>90.1±15.2</td>
<td>88.8±13.2</td>
<td>0.740</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>16.50 (12.77–23.15)</td>
<td>9.90 (8.40–12.18)</td>
<td>0.0005*</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.90 (3.00–4.80)</td>
<td>2.10 (1.70–2.75)</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>25.13±7.07</td>
<td>12.86±2.34</td>
<td>0.0005*</td>
</tr>
<tr>
<td>C-reactive protein (CRP) (mg/L)</td>
<td>3.6 (0.56–12.5)</td>
<td>0.35 (0.23–2.1)</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

Data are presented as means±SD for creatinine, FPG and resistin and as median (25th–75th percentiles) for TGs, total cholesterol, eGFR, insulin, HOMA and CRP

*Statistically significant (p<0.05)

eGFR: Estimated glomerular filtration rate, HOMA: Homeostasis model assessment

**DISCUSSION**

General metabolic alterations in patients with CKD have a profound impact on the biology of adipocytes. CKD is a pathological condition two major hallmarks of which are chronic inflammation and insulin resistance. In this study, significantly higher serum insulin levels and increased insulin resistance index measured by the HOMA formula were noted in patients with CKD, compared with the control subjects (p<0.05). Serum creatinine showed significant positive correlation with serum insulin and with HOMA and eGFR showed significant negative correlation with serum insulin and with HOMA. In accordance with these results, Park and Lindholm reported that uremic
toxins per se are thought to cause an acquired defect in the insulin-receptor signaling pathway, and increased inflammation in uremia further aggravates insulin resistance. Lee and co-workers\textsuperscript{18} showed in a cross-sectional study with nondiabetic patients with end stage renal disease higher insulin resistance assessed by HOMA. In nondiabetic hemodialysis patients, insulin resistance assessed by HOMA was an independent predictor of cardiovascular mortality.\textsuperscript{19}
With regard to resistin, a highly statistically significant difference was detected in the serum level of resistin between patients with CKD and control subjects, and also between patients in different stages of CKD, being higher in stage 5 than stage 4. Significant positive correlation between resistin and creatinine and 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,20 Diez et al,21
and Ziegelmeier et al. reported that various adipokines, including resistin, were significantly elevated in patients with stage 5 CKD compared with control subjects and that the regulation of these adipokines in vivo is strongly dependent on renal function. The close relationship between eGFR and plasma resistin level favors the possibility of reduced filtration of resistin with declining GFR. Nusken et al. suggested that renal function is an important factor in the regulation of the systemic levels of resistin. Risch et al. failed, however, to demonstrate an association between GFR and serum resistin at GFR >60 mL/min/1.73 m², suggesting that resistin level in mildly impaired and normal renal function is influenced by factors other than GFR.

In the present study, significant positive correlation was found between serum levels of resistin and insulin, and between resistin and HOMA. This correlation was separate from the link between resistin and obesity-induced insulin resistance, as obese subjects with BMI >30 kg/m² were excluded from this study. Al-Harithy and Al-Ghamdi found that resistin was correlated with insulin and HOMA in lean, overweight and obese non-diabetic and diabetic subjects. Yaturu et al. 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. reported that resistin may 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. stated that a greater than 5-fold increase in resistin blood levels was not associated with deterioration in insulin sensitivity in patients with renal disease. Filippidis et al. showed that increased serum resistin levels in patients on hemodialysis were not related to the reduced insulin sensitivity encountered in uremia, and Axelsson et al. reported 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.

In this study, no correlation was found between serum levels of resistin and total cholesterol or triglycerides in patients with CKD. These findings are consistent with those of Kielstein et al. and Yaturu et al. In contrast, Taskapan et al. and Park and Lindholm 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.

Inflammation is a common feature that predicts outcome in CKD. Current evidence suggests that inflammation starts early in the process of failure of kidney function, even among patients with moderate impairment in renal function. A sustained state of chronic inflammation is closely linked with several complications of CKD, such as vascular degeneration, myocardial fibrosis, loss of appetite, insulin resistance, increased muscle catabolism and anemia. Evidence of systemic inflammation is a significant predictor of poor outcome in patients with CKD. In this study, on investigating serum level of CRP as a marker of inflammation, CRP was found to be significantly higher in patients with CKD than control subjects. This finding agreed with those of Eustace et al. who reported an association between increasing level of CRP and decreasing eGFR. In addition, Cottone et al. found that plasma concentration of CRP increased in patients with CKD who had left ventricular hypertrophy, but Menon et al. reported no difference in CRP levels. It appears that CKD is possibly associated with risk factors that favor the presence of inflammation rather than being a direct cause of inflammation. In other studies, Kunnari et al. and Shetty et al. also demonstrated a positive correlation between CRP and resistin, independent of BMI, and Cheung et al. showed a significant association between resistin concentration and markers of inflammation. Reilly et al. however, reported that plasma resistin levels were correlated with markers of inflammation and were predictive of coronary atherosclerosis in humans, independent of CRP.

In the present study, when patients with CKD were stratified according to the level of CRP into those with CRP ≤6 and those with CRP >6 ml/L a highly statistically significant difference in serum level of resistin was observed between the two groups. In agreement with these findings, Yaturu et al. found increased levels of resistin and CRP, suggesting a role for resistin as a possible surrogate marker of inflammation in subjects with CKD. Increased CRP levels were related strongly to impaired endothelial dysfunction, coronary artery disease and insulin resistance.

In conclusion, resistin is associated with CKD and its serum level increases with progressive impairment of renal function. Glomerular filtration may represent a crucial metabolic pathway for the elimination of resistin. Resistin is also correlated with CRP in CKD and may be a link with the metabolic and cardiovascular complications of renal disease.
Εκτίμηση της ρεζιστίνης στη χρόνια νόσο των νεφρών

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ΣΚΟΠΟΣ Σύγκριση των επιπέδων ρεζιστίνης του ορού σε ασθενείς με χρόνια νόσο των νεφρών (ΧΝΝ), καθώς και σε φυσιολογικά άτομα, με σκοπό τη διευκρίνιση της σχέσης της ρεζιστίνης με την αντίσταση στην ινσουλίνη και τους δείκτες φλεγμονής στη ΧΝΝ, όπως επίσης και ο ρόλος της ρεζιστίνης στη ΧΝΝ. ΥΛΙΚΟ-ΜΕΘΟΔΟΣ Εξετάστηκαν 60 ασθενείς, ηλικίας 46−70 ετών με ΧΝΝ σύμφωνα με τις οδηγίες της National Kidney Foundation για τη ΧΝΝ και 20 υγιή άτομα ως ομάδα ελέγχου. Αποκλείστηκαν παχύσαρκα και διαβητικά άτομα. Ο εργαστηριακός έλεγχος περιελάμβανε σάκχαρο νηστεία, τριγλυκερίδια, χοληστερίνη, κρεατινίνη, CRP, ρεζιστίνη, ινσουλίνη, υπολογισμό του eGFR και του μοντέλου εκτίμησης της ομοιόστασης (HOMA).

ΑΠΟΤΕΛΕΣΜΑΤΑ Βρέθηκαν στατιστικώς σημαντικά αυξημένα επίπεδα ρεζιστίνης, ινσουλίνης και CRP, καθώς και του HOMA στα άτομα με ΧΝΝ σε σύγκριση με τα άτομα της ομάδας ελέγχου (p<0,05). Τα επίπεδα της ρεζιστίνης ήταν σημαντικά αυξημένα στο στάδιο 5 σε σχέση με το στάδιο 4 της ΧΝΝ (p<0,05). Οι ασθενείς με αυξημένη CRP (>6 mg/L) παρουσίασαν στατιστικά σημαντική αύξηση της ρεζιστίνης σε σχέση με εκείνα που είχαν CRP ≤6 mg/L. Βρέθηκε σημαντικά θετική στατιστική συσχέτιση μεταξύ των επιπέδων ρεζιστίνης και κρεατινίνης του ορού (r=0,856, r=0,302, αντίστοιχα, p<0,05), καθώς και αρνητική συσχέτιση μεταξύ ρεζιστίνης και eGFR (r=-0,285, p<0,05).

ΣΥΜΠΕΡΑΣΜΑΤΑ Η ρεζιστίνη σχετίζεται με τη ΧΝΝ, ενώ τα επίπεδά της στο αίμα αυξάνουν με την επιδείνωση της νεφρικής λειτουργίας και σχετίζεται επίσης με τη CRP, γεγονός που πιθανό να έχει σχέση με μεταβολικές ή καρδιαγγειακές επιπλοκές.

Λέξεις ευρετηρίου: CRP, Ινσουλίνη, Ρεζιστίνη, Χρόνια νεφρική νόσος

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