Predictive Ability Of Neutrophil gelatinase-associated lipocalin and Omentin as markers of Chronic Kidney Disease

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ABSTRACT
Background: Increasing incidence of chronic kidney disease (CKD) which lead to end stage renal disease (ESRD) is one of the major health issues in the modern world and requires novel strategies for treatment. Adipose tissue has been recognized to have endocrine function and secretes a variety of hormones called adipokines. Several adipokines have been implicated in the pathogenesis of CKD and may have a strong impact as a risk factor for renal decline. Aim: To investigate the level of Omentin and Neutrophil gelatinase associated lipocalin (NGAL) in patients with ESRD receiving hemodialysis. Subjects and methods: The present study included 60 patients with end stage renal disease (ESRD) in addition to 30 normal healthy individuals were enrolled in this study as control group. All individuals were subjected to measuring serum level of Omentin, NGAL, creatinine, Ca, Hb, FBS, Na, K, ALT, AST, Alb. Results: This study revealed that there was high significant increase with the mean level of Omentin concentration in ESRD group (488.38 ± 371.08 p< 0.001) compared to healthy group, also there was significant elevation in the mean level of NGAL (902.90 ± 698.35 p< 0.001). Conclusion: Omentin and NGAL suggested to be considered better markers of End Stage Renal Disease.

INTRODUCTION

Kidney diseases are categorized as the highest prevalent ones with worldwide noticeable incidence. They cause accelerated cardiovascular diseases and noticeable mortalities (¹).

The prevalence of end-stage renal disease (ESRD) has been increasing worldwide. It is an important health problem which causes high mortality and morbidity (²). CKD is progressive loss in kidney function over a period of months or years. The symptoms of kidney function are not specific, and might include feeling generally unwell and experiencing a reduced appetite. Often, chronic kidney disease is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with high blood pressure or diabetes and those with a blood relative with CKD. It is differentiated from acute kidney disease (acute kidney injury) in that the reduction...
in kidney function must be present for over 3 months. CKD is an internationally recognized public health problem affecting 5–10% of the world population\(^3\).

Traditional blood markers and urinary markers of kidney injury have been used for decades in clinical studies for diagnostic and prognostic purposes. For prevalent nephropathies such as diabetic kidney disease, they remain as useful clinical markers of renal injury and even of CKD progression. However, considering the variety of causes that lead to kidney damage, these markers seem to be neither sensitive nor specific, mainly in the early disease stages \(^4\).

Additionally, these markers do not directly reflect injury to renal cell signaling pathways and internal organization. Unlike cardiac troponin, most of these markers represent functional and late consequences of injury, and do not explain the type and intensity of aggression that is taking place in the renal parenchyma. Thus, new biomarkers of renal injury and function are required in clinical practice to permit accurate diagnosis, guide treatment, and predict disease progression \(^5\). However, a single biomarker may not be adequate to capture the spectrum of CKD, given the inherent heterogeneity of renal structure and the diverse settings under which kidney injury occurs \(^6\).

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass. Creatinine is a commonly used as measure of kidney function \(^7\).

The creatinine clearance test is used to monitor the progression of renal disease. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the “normal” interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules \(^8\).

Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty acid chain of the other lipids. Through the interaction with the phospholipid fatty acid chains, cholesterol increases membrane packing, which reduces membrane fluidity \(^9\).

Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. It normally constitutes about 60% of human plasma protein, being synthesized primarily by hepatic parenchymal cells except in early fetal life, when it is synthesized largely \(^10\).

The serum enzymes of patients with ESRD are commonly abnormal. This is due in part to the absence of renal excretion and to the frequent presence of multiple comorbid conditions. The serum enzymes most commonly used to assess the diagnosis of hepatobiliary disorders include the alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). ALT and AST are routinely measured to assess liver functions in patients with and without renal failure \(^11\). Omentin, is a recently identified novel adipocytokine of 313 amino acids, which is expressed in visceral (omental and epicardial) fat as well as mesothelial cells, vascular cells, airway goblet cells, small intestine, colon, ovary, and plasma \(^12\). Omentin is the major...
circulating form of omentin and its biological role is still not well known (13).

NGAL (also known as human neutrophil lipocalin, lipocalin-2, siderocalin, 24p3, or LCN) is a small molecule of 25 kDa having 178 amino acids that belongs to the superfamily of lipocalins (14) which are proteins specialized in binding and transporting small hydrophobic molecules. NGAL was originally isolated from the supernatant of activated neutrophils (15) and identified as apolypeptide covalently bound to gelatinase - . NGAL is also normally expressed at very low levels in several human tissues, including the kidneys, lungs, stomach, and colon (16).

Injured kidney tubular cells produce and secrete several biological substances associated with innate and acquired inflammatory immune responses, including NGAL (17).

NGAL was one of the top upregulated genes in damaged kidneys and fulfilled the criteria of a promising biomarker of tubular damage following the start of acute kidney injury. NGAL is systemically synthesized in response to kidney damage. Hence, NGAL appears to deliver a ‘real-time’ assessment of tubular injury. Depending on finding in mice and patients, a model of NGAL trafficking across the nephron was developed (18). The circulating NGAL is freely filtered, under physiological conditions, through the renal glomeruli (as a protein with low-molecular-weight and positive charge) and reabsorbed in the proximal tubule. Thus low levels of plasma NGAL is predictable in the absence of kidney disease (19).


This study was conducted on 90 Egyptian individuals classified into two groups as the following: groups 1 consist of 60 patients with End stage renal disease from Urology center, Mansoura University, and groups 2 consist of 30 healthy individuals with no history of kidney disease.

Blood samples were collected from all patients and healthy control group. Common biochemical parameters, including Creatinine, Ca, Hb, FBS, Na, K, Albumin, ALP, ALT, AST and Cholesterol were measured at baseline in all patients, according to standard methods in the routine clinical laboratory.

Estimation of serum creatinine, albumin, cholesterol, and hemoglobin concentration were performed by colorimetric method according to (23), (24), (25), (26) respectively.

Estimation of serum aspartate transaminase (AST), serum alanine transaminase (ALT) and Alkaline Phosphatase (ALP) activities were determined by colorimetric method according to (27), (28), (29) respectively.

NGAL and Omentin were determined by ELISA according to No.201-12-1720, 201-12-0156 kits respectively.

Statistical Analysis

All statistical analyses were done by a statistical for social science package “SPSS” 14.0 for Microsoft Windows, SPSS Inc and considered statistically significant at a two – sided P<0.05 (30).

Numerical data were expressed as mean ± SD. The levels of markers were analyzed by ANOVA. The correlations between serum biochemical data in different studied groups were evaluated by Person’s correlation coefficient, to
quantify the relationship between the studied parameters. P value < 0.01 was considered significant.

RESULTS

The mean value of Creatinine in negative control was found to be 0.58±0.13 and this was increased to 9.84±3.34 (p<0.001) in ESRD patients. The mean value of K in negative control was found to be 4.14±0.43 and this was increased to 5.10+_0.69 (p<0.01). The mean value of PO₄ in negative control was found to be 3.43±0.64 and this was increased to 4.80±1.69 (p<0.001) in ESRD patients. The mean value of ALP in negative control was found to be 80.83±23.45 and this was increased to 195.91±132.19 (p<0.001). The mean value of albumin in negative control was found to be 3.95±0.24 and this was decreased to 3.59±0.31 (P<0.001) in ESRD patients. The mean value of Hb% in negative control was found to be 14.48±1.28 and this was decreased to 11.0±1.32 (P<0.001). The mean value of Ca in negative control was found to be 9.85±0.52 and this was decreased to 8.01±0.82 as shown in Table (1).

Omentin was increased in ESRD patients compared with healthy control group Mean ± SD .488.38±371.08 and 78.71±121.56 (P<0.001), NGAL was increased in ESRD patients compared with healthy control group Mean±SD .902.90±698.35 and 147.99±128.24 (P<0.001) as shown in Table 2.

In this study, Receiver Operating Characteristic (ROC) analysis in ESRD patients revealed that serum NGAL continued to be the best predictor of CKD with a sensitivity of %100 and specificity of %80 with Auc of %0.957 and Omentin showed a sensitivity of %95 and specificity of %60 with Auc %0.913 as shown in fig (1).

DISCUSSION

There is a concern in the nephrology community about strategies for early identification of kidney damage in patients at risk of developing CKD. CKD affects more than 10% of the general population in the U.S. alone and is associated with high rates of all-cause and cardiovascular mortality, imposing a high cost to the public health services. Serum and urinary biomarkers that have been employed for decades to detect renal dysfunction and damage, such as creatinine and proteinuria respectively, cannot capture accurately the extension of the injury within the glomerular, tubular, or interstitial compartments, nor can they predict disease progression, renal survival, and mortality. In this scenario, novel biomarkers of CKD that could reflect early kidney damage and thus provide a window for early clinical intervention have been sought intensively (31).

Serum liver enzymes of patients with end-stage renal disease (ESRD) are commonly abnormal. This is due in part to the absence of renal excretion and to the frequent presence of multiple conditions. Serum enzymes most commonly used to assess the diagnosis of hepatobiliary disorders include the alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). ALT and AST activities may be lower in ESRD patients because of a deficiency in vitamin...
B6, which is a coenzyme of ALT and AST (32).

Total cholesterol was assayed in serum of patients with ESRD and the results indicated that there was a significant decreased (P < 0.05) in patients compared with control group which was (184.43 ± 42.37 mg/dl vs. 187.56 ± 38.27 mg/dl). These results were similar to that found by (33). The decrease of cholesterol concentration could be due to increased hepatic syndrome defective triglyceride removal (34).

Omentin is a novel hydrophilic adipokine of 313 amino acids (35 kDa), which contains a secretory signal sequence and a fibrinogen-related domain, and appears as a glycolized trimer of 120 kDa molecular weight in its negative form (35). Omentin is an important molecule that connects organs with adipose tissue and exerts extensive protective effects (36). Omentin has 3 main functions that have been identified first increases the insulin sensitivity in human adipocytes ,second reduces inflammation and third stimulates vasodilatation (37).

In this study Omentin was increased in ESRD patients compared with healthy control group Mean ± SD .488.38±371.08 and78.71±121.56 (P<0,001) this was in agreement with Al Celik who show significant elevation of serum omentin in End Stage Renal Disease . The reason of increased level of omentin related to impaired renal clearance , and defective degradation and excretion, and this agreed with few studies which found higher levels of omentin in end stage renal disease patients. This may due to the size of omentin which is relatively large protein, which during hemodialysis may not be cleared from plasma most adipokines are elevated in patients with chronic kidney disease (38). Sengul et al reported that serum omentin level was elevated in ESRD patients compared with healthy control group(39).

NGAL was small 25-kD protein, belonging to the “lipocalins” superfamily, is massively released in blood from injured tubular cells after various conditions potentially detrimental to the kidney in experimental and human clinical models. No less important, NGAL release from renal tubule occurs soon after damage, notably preceding the rise in serum creatinine and thus allowing the initiation of preventive therapeutic measures in a timely manner (40). The NGAL molecule is freely filtered by the glomerulus and reabsorbed in the proximal tubules; its secretion in urine occurs after direct renal insults. In chronically damaged tubular cells, high quantities of NGAL are produced due to intracellular stress and protein overload, perhaps as a compensatory response to counteract intracellular complement-induced oxidative stress (41). In this study NGAL was increased in ESRD patients compared with healthy control group Mean±SD .902.90±698.35 and147.99±128.24 (P<0,001) this was in agreement with(42). who show significant elevation of serum NGAL in End Stage Renal Disease because of increasing distal renal tubular expression and synthesis of NGAL and NGAL secretion increasing .In a study conducted by Ozkan et al serum NGAL was elevated in ESRD patients compared with healthy control group(43).

CONCLUSION
It was found that NGAL and omentin levels were elevated in patients with ESRD.NGAL and Omentin were useful
markers for detection chronic kidney disease.

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REFERENCES


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Table (1) Clinical and hematological characteristics of all studied groups.

<table>
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<tr>
<th>Test</th>
<th>Groups</th>
<th>P value</th>
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<tr>
<td></td>
<td>Healthy (I) (N=30) mean±SD</td>
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<tr>
<td>Creat (mg/dl)</td>
<td>0.58± 0.13</td>
<td>&lt;0.000</td>
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<tr>
<td>FBS (mg/dl)</td>
<td>98.80± 26.32</td>
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<tr>
<td>Na (mEq/L)</td>
<td>137.43± 2.0</td>
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<tr>
<td>K+ (mg/dl)</td>
<td>4.14± 0.43</td>
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<tr>
<td>Ca (mg/dl)</td>
<td>9.85± 0.52</td>
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<tr>
<td>PO (mg/dl)</td>
<td>3.43± 0.64</td>
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<tr>
<td>Hb% (g/dl)</td>
<td>14.48± 1.28</td>
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<td>ALP (U/L)</td>
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<td>ALB (g/dl)</td>
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<td>ALT (U/L)</td>
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<td>AST (U/L)</td>
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<td>Chol. (mg/dl)</td>
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<tr>
<td>Creat (mg/dl)</td>
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<td>FBS (mg/dl)</td>
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<td>Na (mEq/L)</td>
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<td>K+ (mg/dl)</td>
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<td>ALT (U/L)</td>
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<td>AST (U/L)</td>
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<td>Chol. (mg/dl)</td>
<td>184.43± 42.37</td>
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Results are presented as mean ± SD

Table (2). Omentin-1 and NGAL in all studied groups.

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<td>Omentin-1</td>
<td>Healthy (I) (N=30) mean±SD</td>
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<tr>
<td>(ng/ml)</td>
<td>78.71± 121.56</td>
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<tr>
<td></td>
<td>ESRD (II) (N=60) mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>488.38± 371.08</td>
<td></td>
</tr>
<tr>
<td>NGAL</td>
<td>Healthy (I) (N=30) mean±SD</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>147.99± 128.24</td>
<td></td>
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<tr>
<td></td>
<td>ESRD (II) (N=60) mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>902.90± 698.35</td>
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</tbody>
</table>

Results are presented as mean ± SD, P-value < 0.05 is considered significant, P-value < 0.01 is considered high significant, P-value < 0.001 is considered very high significant.
Fig (1) ROC curve for serum NGAL and serum omentin levels (ng/ml) in ESRD patients
Our study found that NGAL concentration showed significant positive correlations with omentin, creatinine, K, PO4, ALP, and significant negative correlations with FBS, Na, Ca, Hb, Cholesterol, Alb, ALT, and AST as shown in Fig(2).

Fig(a): correlation between NGAL&OMENTIN
Fig(b): correlation between NGAL&CREAT
Fig(c): correlation between NGAL&NA
Fig(d): correlation between NGAL&FBS
Fig(e): correlation between NGAL&HB
Fig(f): correlation between NGAL&PO4
Fig(g): correlation between NGAL&K
Fig(h): correlation between NGAL&CA
Fig(i): correlation between NGAL&CHOL
Fig(j): correlation between NGAL&AST
Fig(k): correlation between NGAL&ALP
Fig(l): correlation between NGAL&ALB

Fig(2): correlations between all studied parameters