# Neutrophil Gelatinase-Associated Lipocalin as a Novel Biomarker for Chronic Kidney Disease

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By
***Suzan Eid El-Shishtawy Ibrahim***
M.B., B.Ch.
Ain Shams University

**Supervised by**

**Professor / *Ola Hamdy El-Demerdash***
Professor of Clinical and Chemical Pathology
Faculty of Medicine- Ain Shams University

**Professor / *Sayeda Abd El-Raheim Saleh***
Professor of Clinical and Chemical Pathology
Faculty of Medicine- Ain Shams University

**Doctor/ *Osama Mahmoud Mohammed***
Assistant Professor of Internal Medicine
Faculty of Medicine- Ain Shams University

Faculty of Medicine
Ain Shams University
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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AKI | : Acute kidney injury |
| ANOVA | : Analysis of variance |
| Apo D | : Apolipoprotein D |
| ASOT | : Antistreptolysin O titre |
| AUC | : Area under the curve |
| BUN | : Blood urea nitrogen |
| C8 | : Complement 8 |
| CBC | : Complete Blood Picture |
| CKD | : Chronic kidney disease |
| CMV | : Cytomegalovirus |
| CRBP | : Cellular retinoid-binding proteins |
| CrCl | : Creatinine clearance |
| CRF | : Chronic renal failure |
| eGFR | : Estimated GFR |
| ELISA | : Enzyme- linked immunosorbent assay |
| EPO | : Erythropoietin |
| ESR | : Erythrocyte sedimentation rate |
| ESRD | : End-stage renal disease |
| FN | : False negative |
| FP | : False positive |
| GFR | : Glomerular filtration rate |
| GLDH | : Glutamate Dehydrogenase |
| Glu T4 | : Glucose transporter 4 |
| HDL | : High-density lipoprotein |
| HIV | : Human immunodeficiency virus |
| Ht | : Height |
| K/DOQI | : Kidney Disease Outcomes Quality Initiative |
| LMW | : Low molecular weight |
| MAC | : Membrane attack complex |
| MDRD | : Modification of diet in renal disease |
| MMP-9 | : Matrix metalloproteinase- 9 |
| NAD | : Nicotine amide dinucleotide |
| NAG | : N-acetyl-β-D-glucosaminidase |
| NGAL | : Neutrophil gelatinase-associated lipocalin |

| | |
|---|---|
| NKF | : National Kidney Foundation |
| PBS | : Phosphate buffered saline |
| Pcr | : Plasma creatinine. |
| PGDs | : Prostaglandin D synthases |
| PP14 | : Pregnancy Protein 14 |
| RBP | : Retinol-binding protein |
| ROC | : Receiver-operating characteristic |
| SDS-PAGE | : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| TTR | : Transthyretin |
| U NGAL | : Urinary NGAL |
| Ucr | : Urine creatinine. |
| VEGP | : Von Ebner's gland protein |
| WHO | : World Health Organization |

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# INTRODUCTION

Chronic kidney disease (CKD) is a devastating illness with an incidence and prevalence rapidly approaching epidemic proportions worldwide ***(El-Nahas and Bello, 2005).*** It can be caused by a variety of diseases, among which glomerulonephritis especially IgA nephropathy, obstructive uropathy and diabetic nephropathy are the most common ***(Malyszko et al., 2008).***

According to the current Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines, CKD is classified into five stages based on creatinine-based equations for estimating glomerular filtration rate (GFR) ***(National Kidney Foundation K/DOQI, 2002).*** These stages are:

Stage 1: Kidney damage with normal or increased GFR (GFR≥90 mL/min).
Stage 2: Mildly decreased GFR (GFR 60-89 mL/min).
Stage 3: Moderately decreased GFR (GFR 30-59 mL/min).
Stage 4: Severely reduced GFR (GFR 15-29 mL/min).
Stage 5: kidney failure (GFR < 15 mL/min or dialysis).

Early intervention can significantly improve the prognosis. However, lack of sensitivity and imprecision of the currently available biomarkers has impaired the ability to initiate potentially effective therapies in a timely manner ***(Devarajan, 2008).***

Neutrophil gelatinase-associated lipocalin (NGAL, also known as human neutrophil lipocalin, lipocalin-2, siderocalin, 24P3 or LCN2) is a 25-Kd protein that belongs to the well-defined superfamily of proteins called lipocalins ***(Goetz et al., 2002)***.It was originally identified as a component of neutrophil granules ***(Kjeldsen et al., 2000)***. It is also expressed at a low level in other tissues including the kidney ***(Schmidt-Ott et al., 2007)*.** NGAL is synthesized systemically in response to kidney damage, followed by glomerular filtration and tubular uptake, and it could be produced locally by injured tubules. A third source of NGAL may be the activated neutrophils, macrophages or inflamed vasculature, frequently found in chronic kidney disease. This indicates that NGAL may be a promising biomarker for kidney damage ***(Devarajan, 2008)***.

# AIM OF THE WORK

The aim of the present work is to study serum neutrophil gelatinase-associated lipocalin (NGAL) in a group of patients with different stages of chronic kidney disease in order to evaluate its clinical utility in diagnosis as well as assessment of disease severity.

# I. CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is defined as the presence of objective kidney damage and/or the presence of glomerular filtration rate(GFR) of 60 mL/min/1.73 m² body surface area or less for at least three months irrespective of the underlying etiology of the kidney damage ***(Graves, 2008)***.

Evidence of kidney damage may be either structural or functional in nature and may be derived from renal histology or from the results of appropriate urine, blood or renal imaging studies. The presence of abnormal sediment on urine microscopy or the demonstration of multiple cysts on renal imaging in a patient with a family history of polycystic kidney disease would meet the requirement for objective kidney damage ***(Fadrowski et al., 2006)***.

## A. Incidence of Chronic Kidney Disease:

Chronic kidney disease is a world-wide public health problem. It is recognized as a common condition that is associated with an increased risk of cardiovascular disease and chronic renal failure (CRF). More than 50 million people world-wide have chronic kidney disease, and more than one million of them are receiving kidney replacement therapy. Early detection may help slow the progression of kidney disease and avoid kidney failure. Most people with chronic kidney disease do not die of kidney failure, they die of heart disease. In fact, heart disease causes 40-50% of all deaths in patients with chronic kidney disease ***( Schoolwerth et al., 2006)***.

## B. Pathophysiology of Chronic Kidney Disease:

Approximately one million nephrons are present in each kidney, each contributing to the total GFR. Regardless of the etiology of renal injury, with progressive destruction of

nephrons, the kidney has an innate ability to maintain GFR by hyperfiltration and compensatory hypertrophy of the remaining healthy nephrons. This nephron adaptability allows for continued normal clearance of plasma solutes so that substances such as urea and creatinine start to show significant increases in plasma levels only after total GFR has decreased to 50%. When the renal reserve has been exhausted, the plasma creatinine value will approximately double with a 50% reduction in GFR. A rise in plasma creatinine from a baseline value of 0.6 mg/dL to 1.2 mg/dL in a patient, although still within the reference range, actually represents a loss of 50% of functioning nephron mass. The residual nephron hyperfiltration and hypertrophy, although beneficial for the reasons noted, has been hypothesized to represent a major cause of progressive renal dysfunction. This is believed to occur because of increased glomerular capillary pressure, which damages the capillaries and leads initially to focal and segmental glomerulosclerosis and eventually to global glomerulosclerosis ***(Matyus et al., 2008)***.

Factors that may cause progressive renal injury include the following; systemic hypertension, acute insults from nephrotoxins or decreased perfusion, proteinuria, increased renal ammoniagenesis with interstitial injury, hyperlipidemia and hyperphosphatemia with calcium phosphate deposition ***(Polzien, 2007***).

## C. Etiology of Chronic Kidney Disease:

### 1- Diabetic Nephropathy:

Diabetes mellitus is a state of chronic hyperglycemia sufficient to cause long-term damage to specific tissues, notably the retina, kidneys, nerves and arteries. It affects 176 million people world-wide and the World Health Organization (WHO) predicts that the prevalence of diabetes is set to double by 2030. Type 1 diabetes is due to autoimmune destruction of the

insulin-secreting cells of pancreatic β-cells. Type 2 diabetes is due to the combination of cellular resistance to insulin and beta cell failure. Tissue lesions are common to both types of diabetes, and chronic hyperglycemia or a closely related metabolic abnormality is responsible for diabetic complications including diabetic nephropathy ***(Rigalleau et al., 2008)***.

Diabetic nephropathy is a clinical diagnosis based on the finding of proteinuria in a patient with diabetes and in whom there is no evidence of urinary tract infection. Overt nephropathy is characterized by protein excretion greater than 0.5 g/day. This is equivalent to albumin excretion of around 300 mg/day. It is preferable to assess proteinuria as albuminuria because it is a more sensitive marker for CKD due to diabetes. The NKF-K/DOQI Work Group concluded that urinary albumin should be measured to detect and monitor kidney damage in adults. Patients are considered to have microalbuminuria when the urinary albumin excretion rate is between 30 and 300 mg/day ***(Zerbini et al., 2006)***.

In patients with type 1 diabetes, the microalbuminuria will progress to overt nephropathy at an average rate of 20% over 5 years ***(Rigalleau et al., 2008)***. Since the onset of type 2 diabetes is difficult to define, it is difficult to estimate the incidence of microalbuminuria. As albuminuria worsens and blood pressure increases, there is relentless decline in GFR. In some patients with microalbuminuria, renal lesions are already quite advanced and therefore, it may be a marker of nephropathy rather than a predictor of renal structural changes ***(Zerbini et al., 2006)***.

There may be a genetic predisposition to develop diabetic nephropathy. Genetic determinants and their impact on the initiation and progression of diabetic nephropathy continue to be actively investigated. Numerous metabolic pathways and associated groups of genes have been proposed as candidates to

play a role in the genetic susceptibility to nephropathy ***(Murphy et al., 2008)***.

**2- Hypertension/Ischemic Kidney Disease:**

Hypertension is the second most common attributed etiology of CKD in the world. There is abundant evidence that hypertension, especially systolic hypertension, is a powerful promoter of kidney damage. It may exacerbate the renal injury and rate of decline that occurs from a given disease ***(Hausberg et al., 2008)***.The relationship between hypertension and CKD is difficult to establish because hypertension is a frequent consequence of CKD and thus is likely to be present in a large proportion of subject with CKD regardless of their initial etiology ***(Padwal et al., 2008)***.However, there is also clear evidence that hypertension predates an increased risk of end-stage renal disease (ESRD). In addition, control of blood pressure clearly decreases the risk of CKD progression ***(Rao et al., 2008)***.

Renal artery stenosis is one of the important causes of renal vascular hypertension; it causes fibromuscular hyperplasia which commonly occurs in women under 50 years old. The remainder of renal vascular disease is due to atherosclerotic stenosis of the proximal renal arteries ***(*Lee *et al., 2006)***.The mechanism of hypertension is excessive renin release due to reduction in renal blood flow and perfusion pressure. Renal vascular hypertension may occur when a single branch of the renal artery is stenotic, but in as many as 25% of patients both arteries are obstructed ***(Hausberg et al., 2008)***.

**3- Post-Infectious Glomerulonephritis:**

Post-infectious glomerulonephritis is often associated with post-streptococcal infections due to nephritogenic group A beta-hemolytic streptococci, especially type 2. It commonly appears after pharyngitis within one week after infection. Other causes of post-infectious glomerulonephritis include bacteremic

states such as systemic staphylococcus aureus infection, infective endocarditis and shunt infections ***(Blyth et al., 2007)***.

Patients with post-infectious glomerulo-nephritis complain of oliguria, generalized oedema and variable hypertension. Serum complement levels are low; antistreptolysin O titres (ASOT) can be high unless the immune response had been blunted with previous antibiotic treatment. Classically, the urine is described as cola-colored. Urinary red blood cells, red cell casts, and proteinuria under 3.5 g/day is present. Immunofluorescence shows IgG and C3 in granular basement membrane. Electron microscopy shows large, dense sub-epithelial deposits ***(Srisawat et al., 2006)***.

**4- Berger's Disease (lgA Nephropathy):**

Berger's disease (lgA nephropathy) is a primary renal disease of IgA deposition in the glomerular mesangium. The inciting cause is unknown, but the same lesion is seen in Henoch-Schonlein purpura. IgA nephropathy is also associated with hepatic cirrhosis, celiac disease, and infections such as human immunodeficiency virus (HIV) and cytomegalovirus (CMV) ***(Lau et al., 2005)***.

This disease is characterized by hypertension, persistent microscopic hematuria, glomerulosclerosis, abnormal renal function and the most unfavorable prognostic indicator is proteinuria > 1 g/day. Serum IgA level is increased in up to 50% of patients, and for that reason a normal serum IgA does not rule out the disease. Serum complement levels are usually normal. Renal biopsy is the standard for diagnosis. It shows a focal glomerulonephritis with diffuse mesangial IgA deposits and proliferation of mesangial cells in the glomeruli. IgG and complement (C3) can also be seen in the mesangium of all glomeruli ***(Lau et al., 2005)***.