Study of Neutrophil Gelatinase-associated Lipocalin in patients with chronic kidney disease

Thesis

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Presented by

Moataz Fatthy Mohammed

M.B.B.Ch.

Under supervision of

Dr. Dawlat Belal

Professor of Internal Medicine

Faculty of Medicine

Cairo University

Dr. Seham Bakry

Assistant Professor of Internal Medicine

Faculty of Medicine

Cairo University

Dr. Iman Obaia

Assistant Professor of BioChemistry

Faculty of Medicine

Cairo University

Faculty of Medicine

Cairo University

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Abstract

Clinical nephrology is discovering neutrophil gelatinase-associated lipocalin (NGAL), a small 25-kDa protein belonging to the lipocalin family, as one of the most promising biomarkers in the diagnostic field of acute kidney injury also suggesting NGAL production from tubular cells may reflect the entity of active renal damage that underlies the chronic impairment condition. In this study, we are aiming to highlight the role of NGAL in assessment the severity of renal impairment. For this reason serum-NGAL and urinary-NGAL was evaluated in a cohort of chronic kidney diseased patients with special consideration to different stages of chronic renal impairment in order to verify the relationship with the severity of renal impairment. Our results showed that in CKD patients' serum-NGAL, urinary-NGAL and the fractional excretion of this protein were notably increased as compared to controls and there was a statistical significant variation with each stage of chronic renal impairment.

Key words:

- Chronic Kidney Disease
- NGAL
- Biomarkers

Contents

List of abbreviations

AAV	**Acute kidney injury**
ACE	**Angiotensin convering enzyme**
AKI	**ANCA associated vasculitis**
ARF	**Acute renal failure**
BMI	**Body mass index**
CAPD	**Continuous ambulatory peritoneal dialysis**
C-ANCA	**Cytoplasmic pattern antineutrophil cytoplasmic antibody**
CKD	**Chronic kidney disease**
CrCl	**Creatinine clearance**
CRF	**Chronic renal failure**
CRP	**C-reactive protein**
DIAS Bp	**Diastolic blood pressure**
ESR	**Erythrocyte sedimentation rate**
ESRD	**End stage renal disease**
F.E	**Fractional excretion**
GFR	**Glomerular filtration rate**
GI	**Gastrointestinal**
HbA1c	**Glycated hemoglobin**
HBV	**hepatitis B virus**
HCV	**hepatitis C virus**
HD	**Hemodialysis**
HDL	**High density lipoprotiens**
HIV	**Human immunodeficiency virus**
HUS	**Hemolytic-uremic syndrome**
IE	**Infective endocarditis**
K/DOQI	**The Kidney Diseases Outcomes Quality Initiative**
LDL	**Low density lipoprotien**
LIF	**Leukemia inhibitory factor**
MDRD	**Modification of Diet in Renal Disease**
MMP9	**Matrix metalloproteinase-9**
NGAL	**Neutrophil gelatinase-associated lipocalin**
NHANES	**Third National Health and Examination Survey**
NSAIDs	**Nonsteroidal anti-inflammatory drugs**
P-ANCA	**Perinuclear pattern antineutrophil cytoplasmic antibody**

PCI	**Percutaneous coronary intervention**
PTH	**Parathyroid hormone**
RBC	**Red blood cell**
SCr	**Serum creatinine**
sNGAL	**Serum Neutrophil gelatinase-associated lipocalin**
SYS Bp	**Systolic blood pressure**
TG	**Triglycerides**
TLC	**Total leucocytic count**
TTP	**Thrombotic thrombocytopenic purpura**
UCr	**Urinary creatinine**
uNGAL	**Urinary Neutrophil gelatinase-associated lipocalin**
USRDS	**US Renal Data System**
VCUG	**Voiding cystourethrogram**
WBC	**White blood cell**

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Introduction

Clinical nephrology is discovering neutrophil gelatinase-associated lipocalin (NGAL), a small 25-kDa protein belonging to the lipocalin family, as one of the most promising biomarkers in the diagnostic field of (AKI) acute kidney injury **(Bolignano D et al., 2008)**

This protein, initially found in activated neutrophils as an innate anti-bacterial factor, is released massively from kidney tubular cells after harmful experimental stimulations of various natures, activating specific iron-dependent pathways with the self-defensive intent to contrast oxidative stress and cellular apoptosis**. (Mori K et al., 2007).**

In patients undergoing treatments potentially detrimental to the kidney, such as contrast medium administration and cardiac surgery (**Hirsch R et al., 2007) (Mishra J *et al.,* 2005)**, as well as in subjects with unstable nephropathies (**Trachtman H *et al.,* 2006)**, the increase in NGAL levels is a good predictor of a brief-term onset of AKI, notably anticipating the resulting increase in serum creatinine levels and thus enabling the arrangement of preventive therapeutic measures in a timely manner. In parallel, recent studies have also reported altered NGAL levels in patients affected by some chronic kidney disease (CKD) associated conditions, such as autoimmune **(Brunner HI et al., 2006**), polycystic **(Bolignano D *et al.*,2007)** and proteinuric diseases , **(Ding H et al.,2007) (Bolignano D et al., 2008)**, suggesting the possibility that under these circumstances NGAL production from tubular cells may reflect the entity of active renal damage that underlies the chronic impairment condition **(Mori K et al.,2007)**.

Aim of work

In this research, we are hoping to find out a way to follow up those patients with chronic kidney disease in different nephrology clinics so as to prevent the development of end-stage renal disease and possibilities of dialysis and kidney transplantation with their complications. Regarding cost-effectiveness, world governments spend annually billions of dollars in renal replacement therapy that can be saved by encouraging studies dealing with management of pre-dialysis patients.

In this study, we are aiming to highlight the role of NGAL in assessment the severity of renal impairment.

For this reason serum-NGAL and urinary-NGAL was evaluated in a cohort of chronic kidney diseased patients with special consideration to different stages of chronic renal impairement. From this point of view, fractional execretion of NGAL may thus represent the expression of how much active kidney damage lies beneath the overall condition of chronic renal impairment, rather than being a simple marker of decreased filtration such as serum creatinine. Also, we might understand the pathophysiological role of this protein in tubular adaptation to renal damage.

CHAPTER I

Chronic Kidney Disease

Introduction

The Kidney Diseases Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) defines chronic kidney disease (CKD) as either kidney damage or a decreased kidney glomerular filtration rate (GFR) of < 60 mL/min/1.73 m^2 for 3 or more months. Whatever the underlying etiology, the destruction of renal mass with irreversible sclerosis and loss of nephrons leas to progressive decline in GFR.

The K/DOQI definition and the classification of CKD allow better communication and intervention at the different stages. **(K/DOQI clinical practice guidelines for chronic kidney diease 2002) (Coresh J et al., 2003) (Coresh J et al., 2005).**

The different stage of CKD forms a continuum in time; prior to February 2002, no uniform classification of the stages of CKD existed. At that time, K/DOQI published a classification of the stages of CKD, as shown in the following table (1):

CLASSIFICATION OF CHRONIC KIDNEY DISEASE

Table (1):

Chronic Kidney Disease: A Clinical Action Plan			
Stage	Description	GFR ($mL/min/1.73m^2$)	Action*
	At increased risk	≥90 (with CKD risk factors)	Screening CKD risk reduction
1.	Kidney damage with normal or ↑ GFR	≥90	Diagnosis and treatment Treatment of comorbid conditions, slowing progression, CVD risk reduction
2.	Kidney damage with mild ↓ GFR	60-89	Estimating progression
3.	Moderate ↓ GFR	30-59	Evaluating and treating complications
4.	Severe ↓ GFR	15-29	Preparation for kidney replacement therapy
5.	Kidney Failure	<15 (or dialysis)	Replacement (if uremia present)

Epidemiology

The attention being paid globally to chronic kidney disease is attributable to five factors: the rapid increase in its prevalence, the enormous cost of treatment, recent data indicating that overt disease is the tip of an iceberg

of covert disease, an appreciation of its major role in increasing the risk of cardiovascular disease, and the discovery of effective measures to prevent its progression. These factors render chronic kidney disease an important focus of health care planning even in the developed world, but the problems they delineate in the developing world are far more challenging. Some 85 percent of the world's population lives in low-income or middle-income countries, where the clinical, epidemiologic, and socioeconomic effects of the disease are expected to be the greatest.

Data from the United States suggest that for every patient with end-stage renal disease (ESRD), there are more than 200 with overt chronic kidney disease (stage 3 or 4) and almost 5000 with covert disease (stage 1 or 2). Unfortunately, this type of information is lacking for most other countries, so international comparisons must be based on ESRD, rather than chronic kidney disease.

The prevalence of ESRD is influenced by both the number of new patients requiring renal-replacement therapy (incidence) and the number of deaths. Incidence typically reflects the interaction of genetic and environmental factors, as well as the efficacy of primary health care services. Mortality, for its part, is directly related to the technical and organizational competence of programs offering renal-replacement therapy. With the improvement of such therapy, the known prevalence of ESRD continues to increase in most countries: it is currently higher than 2000 per million population in Japan, about 1500 per million population in the United States, and about 800 per million population in the European Union. In developing countries the figures vary, from less than 100 per million populations in sub-Saharan Africa and India to about 400 per million populations in Latin America and more than 600 per million populations in

Saudi Arabia, despite similar rates of incidence in these countries and regions. Thus, prevalence is largely a matter of survival made possible by renal-replacement therapy, which, in turn, is dependent on health care expenditures and economic strength. Figure (1)

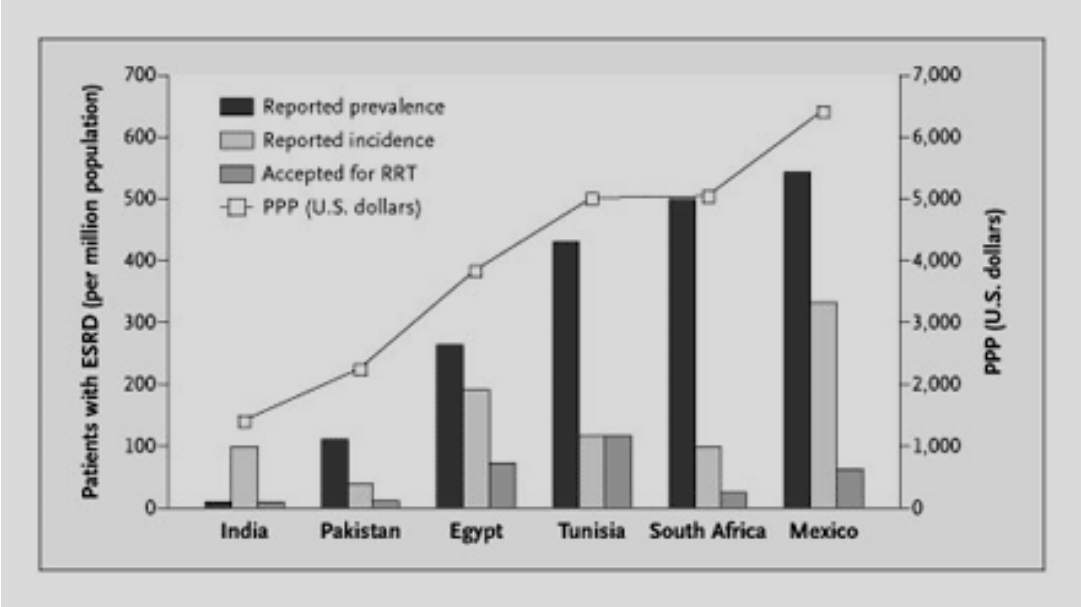

Figure (1) prevalence of ESRD

Although the credibility of statistics from many developing countries may be questionable, the majority of experts agree that 150 per million populations is the average incidence of ESRD. **(Barsoum RS 2002)**

Surprisingly, this figure is lower than those reported in the developed world: in the United States, for example, the incidence is about 330 per million populations. The difference reflects genetic and environmental factors: the role of ethnic origin is evident in the fact that the incidence is much higher among blacks and Hispanics in the United States than among their white counterparts, and the effect of environment is reflected by an