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ORIGINAL ARTICLE

Prognostic significance of NGAL in early stage chronic kidney disease

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ABSTRACT

BACKGROUND: Neutrophilgelatinase-associated lipocalin (NGAL) has been proven to be a useful biomarker for early detection of acute kidney injury, but it is not known whether adding NGAL measurements to conventional risk factors will improve the risk assessment in the setting of chronic kidney disease (CKD). The aim of the present study was to examine the correlation of NGAL with early stage renal impairment in CKD and to evaluate its prognostic value in these subjects.

METHODS: This is a prospective observational cohort study of 54 patients with early stage (stage 1-2) CKD. Patients aged between 18 and 65 years with stable disease were enrolled in this study. Patients with a history of primary glomerulonephritis, diabetes mellitus, acute kidney injury, systemic diseases and stage 3-4-5 CKD were excluded from the study group. Estimated glomerular filtration (eGFR) rate was calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. The patients were followed for two years to determine the ability of baseline NGAL for prediction of renal outcome. In our study disease progression was defined as changes in eGFR (ΔeGFR) and proteinuria (Δproteinuria). Patients divided into two groups according to NGAL cut-off value as group 1 (N.=23, NGAL ≤98.71 ng/mL) and group 2 (N.=31, NGAL >98.71 ng/mL).

RESULTS: Out of 54 patients (mean age: 45.6±7.6 years, 64.8% female, baseline eGFR: 84.6±16.8 mL/min/1.73 m^2, baseline NGAL level: 157.47±121.52 ng/mL); 18 patients were stage 1 and 36 patients were stage 2 CKD. In the ROC analysis, we found that the optimal cut-off value of NGAL for predicting stage 2 CKD was 98.71ng/mL (P=0.005) with the 72.2% sensitivity and 72.2% specificity. In correlation analysis, we evaluated significantly positive correlations between NGAL and CKD stage (r=0.389, P=0.004), baseline/last serum creatinine level (r=0.530, P<0.001 and r=0.439, P=0.003; respectively), last proteinuria level (r=0.359, P=0.043). There were significantly negative correlation between NGAL and baseline/last eGFR (r=-0.498, P<0.001 and r=-0.462, P=0.002; respectively). Compared to the group 1, we determined that group 2 patients had further deterioration in renal functions regarding ΔeGFR (-1.12±12.6 mL/min *vs.* -1.46±12.4 mL/min: respectively, P=0.930) and Δproteinuria (98.1±569.3 mg/day *vs.* 339±701.6 mg/day; respectively, P=0.305); however these differences were not statistically significant at the end of the two years follow-up period.

CONCLUSIONS: Altough NGAL has a positive correlation with disease severity, it does not seem to be a marker of disease progression in patients with early stage CKD. But further studies stated in different patient groups may also explain the usability of NGAL in clinical practice.

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Key words: Lipocalin-2 - Biomarkers - Renal insufficiency, chronic.

Early detection of CKD is important and allows for early commencement of proper therapies to slow down the progression of kidney deterioration. A biomarker of kidney damage which is able to indicate early injury and may identify patients at an increased risk of

progressive disease and may direct treatment has a cardinal importance.[1-3]

Lipocalin-2 or neutrophil gelatinase-associated lipocalin (NGAL) or sidrocalin is a small 25-kilodaltons protein belonging to the lipocalin family, produced by the nephron in response to tubular epithelial damage.[4, 5] It has been well established that NGAL level rises in acute kidney injury (AKI). Recent evidences suggest that NGAL is elevated in CKD, regardless of etiologies, and that elevated NGAL level can predict CKD progression.[6, 7] The aim of the present study was to examine the relation of NGAL with early stage renal impairment in CKD and to evaluate its prognostic value in these subjects.

Materials and methods

This is a prospective observational cohort study recruited from out-patient clinics of Nephrology at Sisli Hamidiye Etfal Training and Research Hospital and from August 2011 to July 2012. The Ethical Review Board of Sisli Hamidiye Etfal Training and Research Hospital approved the study and written informed consent was obtained from the donors and and was conducted according to the tenets of the Declaration of Helsinki.

Subjects aged between 18 and 65 years with stable CKD (defined as fluctuations of serum creatinine <10% in at least three readings during three months) Stage 1 and 2 as per Kidney Disease: Improving Global Outcomes (KDIGO) Guidelines 2012 were enrolled.[8] Patients with a history of malignancy, acute or chronic infections, diabetes mellitus, systemic diseases (such as systemic lupus eritematosus, vasculitis, Sjogren disease, sarcoidosis), primary glomerulonephritis, acute kidney injury and stage 3-4-5 CKD (baseline glomerular filtration rate <60 mL/min/1.73 m^2) were excluded from the study group.

Blood samples were taken in the morning before any food intake. Serum biochemical parameters including urea, creatinine (modified Jaffe method on COBAS 602 modular analyser), 24-hour protein excretion were measured according to the standard methods in the routine clinical laboratory. All of the patients had follow-up visits at the outpatient clinic or by interviews telephonically at monthly intervals, and renal function tests were repeated at 3-month intervals.

Estimated glomerular filtration (eGFR) rate was calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Formula.[9] The staging criteria for CKD were defined as, renal damage with a eGFR at a normal or high level greater than 90 mL/min for Stage 1; renal damage with eGFR of 60-89 mL/min per 1.73 m^2 for Stage 2. The patients were followed for two years to determine the ability of baseline NGAL for prediction of renal outcome. Patients divided into two groups according to NGAL cut-off value; group 1 (N.=23, NGAL≤98.71 ng/mL) and group 2 (N.=31, NGAL>98.71 ng/mL). In our study disease progression was defined as changes in eGFR and proteinuria.

For NGAL measurement blood was placed into chilled vacutainer tubes containing potassium ethylenediamine tetracetate (EDTA) and the plasma was promptly separated by a refrigerated centrifuge (at 4 °C, 5 minute, 4000 x g) and the samples were stored at -20 °C until assessment time. NGAL was evaluated by an enzyme linked immunosorbent assay (ELISA) technique using reagents provided by Quantikine (R&D Systems, Inc, Minneapolis, MN, USA).

Statistical analysis

SPSS 20.0 software (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis. Descriptive statistics were given as mean±standard deviation (SD) and percentage. Yates Correction χ^2 Test were used for comparison of non-parametric variables. Mann Whitney U Test was used for comparison of parametric variables between groups. Spearman test was used for correlation analysis. Receiver operator curve analysis was done for log NGAL and identifying the optimal NGAL cut-off values for predicting basal stage of CKD. The association between basal serum NGAL levels and delta e-GFR was identified by linear regres-

TABLE I.—*Baseline demografic and labrotory characteristics of patients.*

	All patients (N.=54)	Stage 1 CKD (N.=18)	Stage 2 CKD (N.=36)	P value
Demographics				
Age (years)	45.6±7.6	42.2±7.1	47.3±7.3	0.021
Gender (M/F)	19/35	2/16	17/19	0.014
Etiology of CKD (N. %)				
Hypertension	18 (33.3%)	7	11	0.540
Tubulointerstitial nephritis	12 (22.2%)	3	9	0.487
Polycystic kidney diseases	9 (16.7%)	2	7	0.438
Unknown etiology	15 (27.8%)	6	9	0.519
Laboratory				
Baseline urea (mg/dL)	28.9±9.9	25.2±9.5	30.8±9.7	0.054
Baseline creatinine (mg/dL)	0.92±0.21	0.71±0.10	1.03±0.14	<0.001
Baseline e-GFR (mL/min)	84.6±16.8	104.7±7.0	74.6±9.7	<0.001
Baseline albumin (gr/L)	4.2±0.3	4.3±0.3	4.1±0.3	0.074
Baseline proteinuria (mg/day)	246.4±206.7	303.3±236.9	218.1±187.0	0.155

sion model. A P value of <0.05 were considered statistically significant.

Results

Totally 54 patients (Stage 1 CKD, N.=18; Stage 2 CKD, N.=36 patients) were included into the study. Participants predominantly were female (64.8%) with a mean age of 45.6±7.6 years, baseline eGFR: 84.6±16.8 mL/min/1.73 m^2, baseline NGAL level: 157.47±121.52 ng/mL. Hypertension (33.3%), tubulointerstitial nephritis (22.2%), polycystic kidney diseases (16.6 %), and unknown etiology (27.8%) were the leading causes of ESRD. As shown in Table I the plasma NGAL levels, creatinine levels and eGFRs were significantly higher in Stage 2 CKD patients compared to Stage 1 CKD.

In the ROC analysis, we found that the optimal cut-off value of NGAL for predicting Stage 2 CKD was 98.71 ng/mL (P=0.005) with the 72.2% sensitivity and 72.2% specificity (Figure 1). We observed significantly positive correlations between NGAL and CKD stage (r=0.389, P=0.004), baseline/last serum creatinine levels (r=0.530, P<0.001 and r=0.439, P=0.003; respectively), last proteinuria levels (r=0.359, P=0.043) according to correlation analysis (Table II). There were significantly negative correlation between NGAL and baseline and last eGFRs (r=-0.498, P<0.001 and r=-0.462, P=0.002; respectively). There were

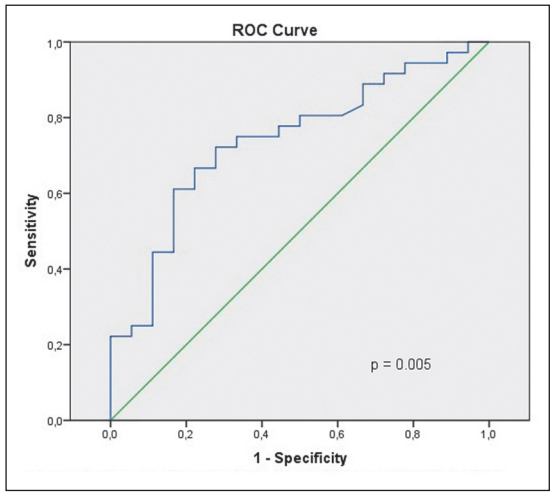

Figure 1.—Sensitivity and specifity of optinmal cut-off value of NGAL in the ROC analysis.

TABLE II.—*Parameters correlated with baseline NGAL.*

Parameters	r	P
Age	0.024	0.861
Gender (male)	0.255	0.063
CKD stage	0.389	0.004
Baseline eGFR (mL/min)	-0.498	<0.001
Baseline urea (mg/dL)	0.029	0.834
Baseline creatinine (mg/dL)	0.530	<0.001
Baseline albumin (g/L)	0.022	0.872
Baseline proteinuria (mg/day)	0.080	0.563
Last eGFR (mL/min)	-0.462	0.002
Δ eGFR (mL/min)	0.041	0.795
Last urea (mg/dL)	0.234	0.135
Last creatinine (mg/dL)	0.439	0.003
Last albumin (g/L)	0.084	0.606
Last proteinuria (mg/day)	0.359	0.043
Δ proteinuria (mg/day)	0.173	0.344

TABLE III.—*Lineer regression analyse of baseline NGAL levels that may predict ΔeGFR.*

	Beta	P	95% Confidence Interval	
			Lower	Upper
Baseline NGAL	0.010	0.948	-0.003	0.003

no correlation between baseline NGAL level and age, gender, serum urea level, baseline proteinuria, changes of (Δ) eGFR and proteinuria (Table III). Compared to the Group 1 (NGAL ≤98.71 ng/mL), we found that Group 2 (NGAL >98.71 ng/mL) patients had further deterioration in renal functions regarding ΔeGFR (-1.12±12.6 mL/min *vs.* -1.46±12.4 mL/min; respectively, P=0.930) and Δproteinuria (98.1±569.3 mg/day *vs.* 339±701.6 mg/day; respectively, P=0.305); however this differences were not statistically significant at the end of the two years follow-up period (Tables IV, V).

Discussion

Plasma NGAL level was significantly higher in Stage 2 CKD patients and had positive correlation with disease severity. But plasma NGAL was not a marker of disease progression in patients with early stage CKD.

In clinical practice, there is always a need for markers that may be useful for early diagnosis, predict progression and complications, influence the treatment strategy and categorize patients for their risks. Serum creatinine level can not identify early renal damage or predict renal damage progression. There is a growing need to find an early marker of renal damage. NGAL is synthesized systemically in response to kidney damage and previous studies suggest that NGAL has the potential to be an ideal biomarker to assign early renal damage in CKD patients and significantly correlated with the severity of renal damage.[10-12] Mitsnefes and his colleagues reported that plasma NGAL correlated better with eGFR than serum creatinine and cystatin C.[13, 14]

Elevated levels of NGAL, as well as an inverse correlation with GFR, have been documented in a number of publications examining patients with CKD. Malyszko J *et al.* investigated NGAL levels in 92 non-diabetic patients with CKD Stages 2 to 4. In multiple regression

TABLE IV.—*Characteristics and comparison of two NGAL groups.*

	NGAL group 1 NGAL ≤98.71 ng/mL (N.=23)	NGAL group 2 NGAL >98.71 ng/mL (N.=31)	P
Age (years)	44.3±7.7	46.5±7.4	0.311
Gender (M/F)	5/18	14/17	0.075
Baseline urea (mg/dL)	26.0±8.9	31.1±10.2	0.067
Baseline creatinine (mg/dL)	0.80±0.16	1.02±0.17	<0.001
Baseline e-GFR (mL/min)	95.0±12.4	76.9±15.6	<0.001
Baseline albumin (gr/L)	4.26±0.33	4.25±0.39	0.934
Baseline proteinuria (mg/day)	256.1±225.2	239.3±195.4	0.770
Last e-GFR (mL/min)	93.3±15.9	74.5±17.6	0.001
Δ e-GFR (mL/min)	-1.12±12.6	-1.46±12.4	0.930
Last urea (mg/dL)	24.6±6.0	31.9±12.6	0.038
Last creatinine (mg/dL)	0.82±0.20	1.04±0.29	0.010
Last albumin (gr/L)	4.40±0.28	4.40±0.34	0.984
Last proteinuria (mg/day)	374.4±454.0	603.7±747.0	0.321
Δ proteinuria (mg/day)	98.1±569.3	339.0±701.6	0.305

TABLE V.—*Comprasions of ΔeGFR and Δproteinuria in two NGAL groups.*

	NGAL group 1 NGAL ≤98.71 ng/mL (N.=23)	NGAL group 2 NGAL >98.71 ng/mL (N.=31)	P value
Δ eGFR((mL/min)	-1.12±12.6	-1.46±12.4	0.930
Δ proteinuria (mg/day)	98.1±569.3	339±701.6	0.305

analysis, predictors of serum NGAL were creatinine (beta value =0.97, P=0.005), cystatin C (beta =0.34, P=0.01), and eGFR (beta value =1.77, P=0.001) (14). David Bolignano *et al.* showed significant inverse correlation between eGFR and NGAL (r=-0.44, r=0.0001),[4] and Fatemeh Gharishvandi *et al.* showed eGFR has a significant inverse correlation with NGAL (r =- 0.593, P<0.001) and creatinine (r=-0.251, P=0.033).[10] We found that the plasma NGAL level was significantly higher in Stage 2 CKD patients and significant correlation with CKD stage, baseline eGFR, last eGFR, baseline serum creatinine, last serum creatinine and last proteinuria. Serum NGAL is an effective biomarker for detecting early-stage renal damage in CKD patients. Furthermore, there is a significant correlation with the severity of renal damage. NGAL should be investigated as a potential early and sensitive marker of kidney damage.

We determined 98.71 ng/mL (P=0.005) with the 72.2% sensitivity and 72.2% specificity as the best cut-off values to predict early stage CKD in our study. Bolignano *et al*. found that the best cut-off level for NGAL was 435 ng/mL (sensitivity 83.9%, specificity 53.8%) for patients with CKD of stages 2 to 4. In study of Daijun Xiang *et al.*, cut-off value for NGAL levels were 259 mg/L in Stage 4, 317.5 mg/L in Stage 5).[12] In this study cut-off values are lower than other studies because patients in our study group are at early stages of CKD.

Recent evidence suggests that NGAL may even be involved as a mediator of CKD progression. Cross-sectional studies in IgA nephropathy, autosomal dominant polycystic kidney disease, glomerulonephritis, pediatric lupus nephritis and CKD have shown that urinary and serum NGAL are increased across these diseases and may effect disease activity and kidney function.[15] Bolignano D *et al.* reported that urine NGAL or serum NGAL had prognostic value for renal progression, even in CKD patients.

Shen SJ *et al*. identified 92 patients with Stage 2 to 4 CKD caused by primary chronic glomerulonephritis followed for two years. NGAL was significantly correlated with the severity of renal damage and the progression of renal function deterioration.[11] Rhee H *et al.* studied 121 patients with Ig A Nephropathy patients during the median follow-up period of 41.49 months and found that NGAL alone could not predict renal progression, whereas high levels of NGAL did successfully predict renal progression and increased NGAL represents decreased renal function.[16]

We found that elevated levels of NGAL is not corraleted with worsening renal function, as well as has an inverse correlation with last eGFR and positive correlation with last proteinuria. Increases in NGAL levels are not specific to CKD. NGAL measurements may be influenced by a number of co-existing variables such as chronic hypertension, systemic infections, inflammatory conditions, anemia, hypoxia and malignancies.[17, 18] The routine measurements of NGAL in patients with CKD can not be recommended for monitoring progression.

Conclusions

Serum NGAL is an effective biomarker for detecting early-stage renal damage in CKD patients. NGAL was significantly correlated with the severity of renal damage. NGAL measurements may be influenced by a number of co-existing variables so that it may not used as a marker of disease progression in patients with early stage CKD.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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