Assessment of Neutrophil gelatinase-associated lipocalin (NGAL) as an early biomarker for detection of renal impairment in hypertensive patients

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ABSTRACT

Chronic kidney disease (CKD) is probably the most important problem of public health in advanced countries. Kidneys are often damaged as a result of high blood pressure. One of our main concerns in patients with hypertension is early detection of kidney disorders. The routine biomarkers such as creatinine have some limitation for this purpose, however recent studies suggest plasma NGAL to be a better marker. Therefor in this study we assessed the diagnostic value of plasma NGAL and compared it with serum creatinine in hypertensive patients. This study was performed on 42 hypertensive patients and 30 healthy Volunteer, both with normal serum creatinine and urea concentration who referred to Shohada Tajrish Hospital, plasma NGAL were measured subsequently using ELISA method and eGFR was considered as the gold standard method(cut off value of<78ml.min.1.73m^2). mean NGAL level was significantly higher in patients in comparison to control group. The sensitivity and specificity were 96% and 100% respectively for plasma NGAL(≥32.2 ng/ml) compared with 76% and 47% for serum creatinine (>0.97 mg/dl). Our findings indicate that NGAL is a better indicator of kidney impairment in the early stages of CKD as compared with serum creatinine in hypertensive patients.

Keywords: Hypertesion; CKD; kidney function; NGAL; Creatinine

INTRODUCTION

Chronic kidney disease (CKD), is determined by a condition with progressive loss of the ability of the kidneys to maintain normal levels of protein metabolism products (such as urea), water balance and maintain normal blood pressure and hematocrit, electrolytes, and body's acid-base. Based on the definitions, chronic kidney disease, is defined as structural or functional damage of the kidney, lasting for more than three months[1]. Typically, diabetes (97%) and hypertension (95%) are risk factors for chronic kidney disease[2] . About 25% of adults worldwide have hypertension (systolic blood pressure ≥ 140mmHg or diastolic blood pressure≥90mmHg which is confirmed by a doctor at least in two separate occasions.), high blood pressure is a pathological factor of decreased kidney function [3, 4]. Over the past twenty years it has been learned that earlier identification and treatment of kidney disease can result in preventing kidney disease progression. Thus, a biomarker of kidney damage that is able to indicate the presence of both early damage and identify patients at an increased risk of progressive disease would impact kidney disease diagnosis and treatment. Although blood creatinine is commonly used as an index of renal function, creatininemia is primarily a marker of glomerular filtration and cannot be considered an ideal biomarker for the estimation of kidney injury, because it is insensitive and is influenced by muscle mass, gender, race, and medications and is unreliable to diagnose renal tubular injury in the absence of significant reduction in the glomerular filtration rate (GFR)[5, 6]. The main cause that CKD is dangerous, is inability to detect it in its early stages by a routine marker such as creatinine; Finally, the disease can be treated if detected at an early stage. Neutrophil Gelatinase-Associated Lipocalin(NGAL) has recently been proved useful to quantitate CKD[7] and it has the potential to be an ideal biomarker in early detection of CKD. NGAL is a small 25-kD molecule, which belongs to the lipocalin family of proteins [8]; NGAL interacts on the

cell membrane with specific receptors (24p3R and megalin) as a complex with iron-siderophores (Holo-NGAL) or alone (Apo-NGAL). After internalization, Holo-NGAL is able to release the iron it carried into the cytoplasm, leading to iron accumulation and regulating specific iron-dependent gene pathways. Endosomal NGAL captures iron via a hypothetical intracellular siderophore, which is followed by recycling to the extracellular space. NGAL then may be destroyed within the cell or recycled outside as Apo-NGAL. Most protective effects attributed to this protein probably are realized through mechanisms based on iron-dependent gene regulation. Moreover, Apo-NGAL can capture intracellular iron-siderophores and transport these to the extracellular space, thus depriving the cell of its iron reserves. This probably represents the way NGAL exerts strong antibacterial properties and under particular conditions may promote cellular apoptosis. NGAL seems to have more complex activities than just its antimicrobial effect; Induction of NGAL may limit tubular injury by modulating various cellular responses, such as proliferation, apoptosis, and differentiation, but the specific mechanisms for such an action are not well understood yet [9]. massively released from renal tubular cells after various injuring stimuli [10].The main aim of the present study was to examine the eventual predictive value of plasma NGAL measurement for detecting CKD in adult patients with high blood pressure, in comparision with serum creatinine.

MATERIALS AND METHODS

The study was performed on 42 hypertensive patient, that their blood pressure is not well controlled, (systolic blood pressure ≥ 140mmHg or diastolic blood pressure≥90mmHg) mean age of 54.33±8.9 years ,who referred to Shohada Tajrish Hospital and 30 healthy (14 men and 16 women) volunteers mean age 54.7±6.8. To minimize potential confounding factors, patients with chronic disease such as diabetes, liver, cardiovascular disease and elevated serum creatinine and urea were excluded from the study. Patients history was carefully recorded by interview and confirmed by checking patients record, also recording drug prescription. Clinical examination, including assessment of blood glucose, was performed. BP was measured by a doctor at least at two separate occasions and the average value was considered for data analysis. Blood samples were taken in the morning before any food intake. Biochemical parameters including urea, creatinine were measured according to the standard methods in the routine clinical laboratory. eGFR was assessed using Cockcroft and Gault formula:

$$eGFR(eCrCl) = \frac{(140 - age).mass(in\ kg).[0.85\ if\ female]}{72.serum\ creatinine\ (in\frac{mg}{dl})}$$

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 4c,5min,5000rpm) and the samples were stored at -20 c until assessment time. NGAL was evaluated using commercially available ELISA kit (Biovender,Norway).

Statistical analysis

Data were expressed as mean±SD or %. T-test was used for comparison of the means between two groups. To assess correlation between eGFR and other variables Pearson coefficient was performed. Receiver operating characteristics (ROC) analysis was used to calculate the area under the curve for NGAL and sCr to find the best pNGAL, cut-off values for identifying the patient with risk factor for CKD. All results were considered significant if P value was <0.05. Data were analyzed using SPSS software version 18.

RESULTS

This study was performed on 42 high blood pressure patients (10men and 32 women) mean age 54.33±8.89 and 30 healthy individuals (14 men and 16 women) mean age 54.7±6.8. As shown in table 1 the plasma NGAL level, creatinine and eGFR were significantly higher in patients compared to the control group.

Table1. Comparision of the mean serum creatinine , eGFR and NGAL in the patient and control group.

P-Value	Control Group	Patient	Variables
	n=3	n=42	
NGAL	(14.59±3.71)	(124.54±118.67)	<0.001
Cr	(0.97±0.136)	(1.058±0.18)	0.036
eGFR(ml/min/1.73m²)	(90.74±10.38)	(77.73±20.19)	0.001

In this study, using the Pearson correlation coefficient, eGFR correlation with various parameters including plasma NGAL and sCr were measured; Based on the results from the above parameters, eGFR showed significant inverse correlation with NGAL (R = - .593, P <0.001), and creatinine (R = - .251, P = .033). ROC analysis (Fig1) showed an area under the curve(AUC) for sCr and pNGAL of 0.63(95% CI, 0.50 to 0.76) and 0.92(95% CI, 0.87 to 0.98) respectively. pNGAL area was significantly different with respect tothat of Cr . The best cut-off values to predict early stages of kidney disease for plasma NGAL was found to be ≥32.2 ng/ml (sensitivity and specificity were 96% and 100% respectively)whereas for sCr it was>0.97 mg/dl (sensitivity 76% and specificity 47%).As shown in Fig 1, percent under the ROC curve for NGAL is greater than creatinine and it is 99%.

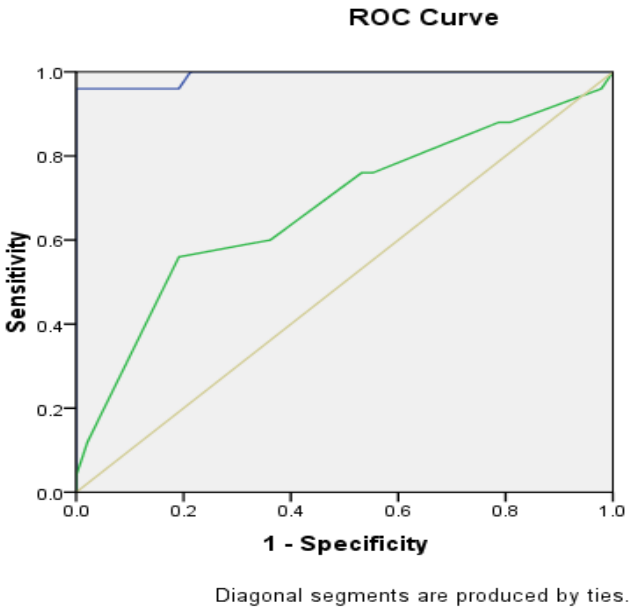

Figure 1. Receiver operating characteristics curves of serum creatinin(sCr),plasma neutrophil gelatinase-associated lipocalin (pNGAL), considering for early detection of CKD as status variable. The area under the curve for sCr, pNGAL 0.63(95% CI, 0.50 to 0.76), 0.92(95% CI, 0.87 to 0.98), respectively. pNGAL was areas significantly different than that of sCr (*P* <0.001). The best cut-off values to predict early stage were determined. For pNGAL this value was found to be 32.2 ng/ml, with a sensitivity of 96% and a specificity of 100%.

DISCUSSION

Early detection and treatment of kidney disease can result in preventing kidney disease progression. Thus, an early biomarker of kidney damage which can identify patients at an increased risk of progressive disease would impact kidney disease diagnosis and treatment[11].

Since creatinine is not a sensitive marker of kidney function and since eGFR also has some limitations, there is a growing need to find an early marker of kidney damage ,new studies suggest that NGAl has the potential to be an ideal biomarker to find early kidney damage in patients at risk [11]. NGAL is synthesized systemically in response to kidney damage. It could be also produced locally by injured tubules. A third source of NGAL may be activated neutrophils/macrophages or inflamed vasculature, frequently found in CKD [12]. In our study we assessed whether NGAL represented a novel, sensitive biomarker of kidney function. We found that mean plasma NGAL level was significantly higher in hypertensive patients compared with the control group this result in consistent with the observation of David Bolignano et al who reported higher mean sNGAL in patients with CKD compared with the healthy control group [13].

Furthermore in this study eGFR showed significant inverse correlation with NGAL (R = - .593, P <0.001), and creatinine (R = - .251, P = .033), in the patients. Where as pNGAL inverse correlation with eGFR displayed higher values compared with sCr. Similar results were observed on univariated analysis by David Bolignano et al that showed significant inverse correlation between eGFR and sNGAL(r=-0.44, P= 0.0001)[13]. In our study, we confirmed finding by Mitsnefes et al. who showed that NGAL could represent a marker of renal function in children with CKD(7). In David B et al research the area under the curve for eGFR and sNGAL was 0.64 (95% CI, 0.53 to 0.73) and 0.70 (95% CI, 0.60 to 0.79) respectively. SNGAL area was statistically different with respect to that of eGFR(*P* < 0.03). In our study ROC analysis indicated that NGAL was a better indicator than sCr for predicting a GFR<78 ml/min.

In our study the best cut-off values to predict early stage were determined. For pNGAL this value was found to be 32.2 ng/ml, with a sensitivity of 96% and a specificity of 100%. In David Bolignano et al the best cut-off level for sNGAL was found to be 435 ng/ml

(sensitivity 83.9%, specificity 53.8%)(13) .In this study Cut-off values are much lower in contrast with previous studies probably because patients in our study are at early stages of CKD. In our subjects, the best overall agreement with eGFR was found for serum NGAL. Moreover, our data suggest that NGAL assay is also able to detect patients with only subclinical or modest renal damage, which may be not revealed by significant variations in renal function test, such as serum creatinine or GFR.

CONCLUSION

Our results indicate that NGAL is a better indicator of kidney impairment in the early Stages of CKD as compared with sCr in hypertensive patients.

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