

Evaluation of some factors affecting the risk of kidney damage in patients with hypertension

Fatemeh Gharishvandi¹, Faranak Kazerouni^{1*}, Ali Rahimipour¹, Malihe Nasiri²

¹Department of Medical Laboratory Sciences, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

²Department of Biostatistics, Faculty of Paramedical Sciences, Tarbiat Modares University, Tehran, Iran.

*Corresponding Author: email address: f_kazerouni@sbmu.ac.ir (F. Kazerouni)

ABSTRACT

Chronic kidney disease (CKD) is an increasing cause of morbidity and mortality worldwide. Prospective data on risk factors for CKD are few. Hypertension is one of the risk factors for CKD. In the past serum creatinine concentration was used as marker of kidney function but it proffers a late reflection of reduced glomerular filtration rate (GFR), which limits its ability to detect impaired kidney function. Cystatin C and NGAL have recently been proven useful to quantitate CKD. Therefore in this study, we assessed the effect of some risk factors on reduction of estimated glomerular filtration rate (eGFR) in patients with high blood pressure. This study was performed on 42 hypertensive patients and 30 healthy volunteers, both with normal serum creatinine and urea concentration. In this study, we measured serum cystatin C and Plasma NGAL. Serum creatinine and urea levels of the patients were measured after an overnight fasting. Estimated glomerular filtration rate (eGFR) was considered as the gold standard method. Serum cystatin C and plasma NGAL were measured using commercially available human ELISA kits. Logistic regression and T-test were used for statistical analysis. The results of logistic regression showed that among the variables studied, plasma levels of NGAL, age and duration of hypertension were significantly associated with the eGFR < 78 (P < 0.05). Our findings suggest that, increased levels of NGAL, age and duration of hypertension predicts a higher odds of impaired renal function.

Keywords: Hypertension; Chronic kidney disease ; Neutrophil gelatinase-associated lipocalin; Cystatin C; Creatinine

INTRODUCTION

Chronic kidney disease (CKD) is increasingly recognized as a global public health problem. CKD is renal injury or decreased kidney function (eGFR) for at least 3 months, as defined by structural or functional abnormalities of kidney, with or without decreased glomerular filtration rate (GFR). Incidence and prevalence of CKD are increasing rapidly, having doubled in past decade due to enhanced longevity of patients with chronic diseases [1, 2]. Extensive studies indicate that chronic kidney disease is a risk factor for other diseases such as: cardiovascular disease [3, 4], anemia [5], electrolyte abnormalities [6], bone disease [7] and cognitive and mental disorders [8]. In the meta-analysis conducted in 2008, it was estimated that the prevalence of chronic kidney disease in persons older than 30 years was about

2.7% and in persons older than 65 years was about 35% [9]. Hypertension is considered to be one of the risk factors of chronic renal failure. Kidneys are the most sensitive organs in the human body that are affected by high blood pressure [10], one complication that affects the kidneys is chronic kidney disease; Hence, early identification of those likely to progress to end-stage renal disease (ESRD) has become important [11]. Serum creatinine (Scr) as a marker of kidney function has already been used, it has been shown that Scr is influenced by factors such as muscle mass, gender and race and eGFRs are less reliable for assessing renal function when GFR is more than 60ml/min. An early marker of kidney damage would promote earlier intervention in order to arrest the progression to end-stage renal disease (ESRD). In order to be of use to the general clinician, the

biomarker must indicate renal damage prior to the current indicators of kidney function, it should be available, non-invasive and easily interpretable [12-14]. Several studies are currently being conducted in this regard with the hope that early identification of kidney disease will lead to early treatment. One example of promising kidney biomarkers is cystatin C [11, 15]. Another potential biomarker is neutrophil gelatinase-associated lipocalin (NGAL) which is a small, robust protein expressed by neutrophils and various epithelia, including the renal proximal tubules. While initially proposed as a marker for infections and certain adenocarcinomas, it is now apparent that its early and dramatic rise in urine after renal injury may make it a useful marker of such injury [16]. Other risk factors such as age, sex [17, 18] and systolic blood pressure [19] are also noted.

MATERIALS AND METHODS

Forty two hypertensive patients who referred to Shohada Tajrish hospital were studied: 10 males and 32 females, mean age was 54.33 ± 8.9 years. We considered subjects to have hypertension if their 24h blood pressure (BP) was ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic. Exclusion criteria included underlying diseases such as diabetes, liver disease, cardiovascular disease, elevated serum creatinine and urea. Normal control blood samples were collected from 30 healthy subjects with mean age 54.7 ± 6.8 without serological abnormality. Control group consisted of 14 males and 16 females. Fasting blood samples were collected from participants; serum and plasma were

separated and frozen until assay. For NGAL measurement blood was placed into chilled vacutainer tubes containing potassium ethylenediamine tetracetate (EDTA). The plasma was promptly separated by a refrigerated centrifuge (at 4°C , 5min, 5000rpm) [20, 21]. The samples were stored at -20°C until assessment time. Plasma NGAL and Scys were measured using an enzyme-linked immunosorbent assay (ELISA) method (Biovender, Norway). Biochemical parameters including urea, creatinines were measured according to the standard methods in the routine clinical laboratory. GFR was estimated from Scr using the Cockcroft and Gault formula [22] and all clearances were expressed as $\text{ml}/\text{min}/1.73\text{m}^2$ after correction for body surface area (BSA) according to the DuBois-DuBois formula [23].

Statistical analysis

Results are expressed as mean \pm standard deviation. P values lower than 0.05 were considered as significant. For comparisons between groups, student's "t" test was used for continuous variables. Logistic regression model was used to adjust the correlations between chronic risk factors for chronic kidney disease in our study and eGFR. Analysis was developed with the statistical package SPSS 18.

RESULTS

Based on the results obtained, as shown in table 1, the pNGAL, Scys levels, diastolic BP, systolic BP and eGFRs were significantly higher in the patients compared to the control group ($p < 0.05$).

Table1. Comparison of the mean pNGAL, Scys, Scr, eGFR, systolic BP, diastolic BP and urea in the patient and the control group.

Variables	Control Group n=30	Patient Group n=42	P-Value
pNGAL (ng/ml)	14.59 ± 3.71	124.54 ± 118.67	<0.001
Scys (ng/ml)	829.27 ± 295.65	1120.9 ± 229.1	<0.05
eGFR ($\text{ml}/\text{min}/1.73\text{m}^2$)	90.74 ± 10.38	77.73 ± 20.19	0.001
Systolic BP	110.5 ± 10.5	$160.5 \pm 18\text{mmHg}$	<0.01
Diastolic BP	60.5 ± 11	$90.7 \pm 9.7\text{mmHg}$	<0.01

Table 2. Determination of the risk of kidney damage according to some of the risk factors in patients with hypertension

Variables	Odds ratio(OR)	95%CI	P-value
Systolic BP	0.76	0.41-1.38	0.37
Duration of disease(year)	1.28	1.03-1.59	0.02
Age(year)	1.32	1.07-1.63	0.009
Sex	9.475	0.73_122.27	0.085
pNGAL(ng/ml)	2.6	1.01-1.2	0.028
Scys (ng/ml)	1	0.99-1	0.5

In this study to determine the relationship between variables such as age, sex, Scys, pNGAL and systolic BP and reduction of renal function, logistic regression was used. Using eGFR <78 as dependent variable in a multiple regression model including all previously reported in analysis, only the association of dependent variable with pNGAL, age, duration of disease remained significant ($p < 0.05$). Table 2 provides a resume of these reports. The odds of incident of renal damage in hypertensive patients was increased by 28%, for each one year increase in duration of disease, the odds of incident of renal damage increased 32% for each one year increase of age. The odds of incident of renal damage was 2.6 times greater for each 10ng/ml increase in pNGAL concentration ($p < 0.05$).

DISCUSSION

Since hypertension is one of the most popular and the most common risk factor of chronic kidney disease [24] in this study, patients with hypertension were selected. In order to determine the odds of reduced renal function in hypertensive patient, pNGAL, Scys and Scr concentrations, age, sex and duration of disease were determined. NGAL early and marked response to the injury (within 2 hours) [25] makes it one of the best markers which up to now, studied. Raised NGAL of renal origin is a direct response to tubule cell injury, while the others are functional markers that may, after a period of time, reveal the effect of such an injury on the accumulation of creatinine and cystatin C in the blood [26]. Cystatin c is produced by all nucleated cells but origin of serum NGAL remains debatable. In this study, we hypothesized NGAL is produced mainly by damaged tubular cells in direct proportion to the degree and severity of the disease [27, 28].

In our study by exclusion of patients with underlying diseases such as diabetes, liver and cardiovascular disease interfering factors, such as activated neutrophils and vascular inflammation were omitted; perhaps this is strength of our study in comparison with other studies. The results of logistic regression to determine the risk of kidney damage indicated that pNGAL was an independent predictor of an unfavorable clinical course and markedly improved the performance of the prediction model, this result is consistent with the observation of David Bolignano et al [20] who reported a significant correlation for NGAL concentrations and age on the risk of kidney injury progression in regression analysis and a strict, independent and inverse correlation of NGAL with estimated GFR, suggesting that under these particular conditions this protein may also represent a surrogate index of residual renal function. Our results are in accordance with results obtained by Colins et al [17] who showed significant correlation between age and duration of disease as the most important risk factor for the loss of renal function. Increased levels of NGAL also predicted higher odds of renal function decrease.

In our study, the systolic BP was not significantly associated with decreased eGFR. In contrast to our study Haroun et al [29] found a significant relationship between gender and decreased renal function inconsistency of our results with the above 2 studies could probably be due to the small size of our study population. Based on our results from multiple regression analysis cystatin C was not as an independent marker associated with eGFR; similarly Mena et al [30] found that cystatin C concentration in hypertensive patients was not correlated with GFR, probably because cystatin C has cardiovascular effects beyond its use as a marker of the renal function. In summary, we

found that pNGAL levels, duration of the hypertension and age clearly correlate with severity of renal impairment, probably expressing the degree of active damage underlying the chronic condition. However, our results should be confirmed by clinical samples from a large longitudinal studies.

CONCLUSION

Our study suggests that NGAL, duration of the hypertension and age may help in stratifying odds of renal dysfunction in patients with hypertension.

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ACKNOWLEDGMENT

The work was resulted from MSc program of Fatemeh Gharishvandi, student of clinical biochemistry. We would like to thank Dr. Hamid Alavimajd faculty member of Shahid Beheshti University of Medical Sciences, for his generous cooperation.

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