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Assessing the Prediction Power of Plasma Neutrophil Gelatinase-Associated Lipocalin and Serum Cystatin C for Diagnosis Kidney Damage

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ABSTRACT

Background & Objective: Chronic Kidney Disease (CKD) has been recognized as a serious public health threat. The early detection of kidney damage in CKD is a useful way to reduce the disease burden. This study aimed to determine the power of Neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (Cys-C) to predict the kidney damage in Iranian patients.

Materials & Methods: This study was conducted at Shohadaye Tajrish Hospital on 72 renal patients. The estimated glomerular filtration rate (GFR) was assumed as the gold standard method. The NGAL and Cys-C were used as predictors and estimated GFR was used as a response variable. Three logistic regression models were fitted to investigate the impact of single and multiple markers for the prediction of GFR status.

Results: The regression models with NGAL and Cys-C as single predictors, and with both of them as multivariate predictors, were fitted to the data. The markers except for Cys-C were significantly related to the renal damage in all models ($P<0.05$). The obtained odds ratio for the model with NGAL, Cys-Cand both NGAL and Cys-C were 1.142, 1.004 and 1.125, respectively. The sensitivity and specificity of the models with NGAL, Cys-C and both of them were 96.00 and 100.00; 64.00 and 97.87; and 96.00 and 100, respectively.

Conclusion: Our findings revealed that the NGAL biomarker as a single predictor could result in high predictor power for classifying the patients with and without kidney damage. Thus, the clinicians can use this marker for the early prediction of this renal problem.

Keywords: Kidney damage, NGAL, Cystatin C, AUC, Iranian population

Introduction

Chronic Kidney Disease (CKD) refers to a decreased renal function, which is a gradual process (1). It can progress to the end-stage kidney after several years. This disease is heavily dependent on glomerular filtration rate (GFR) which is the rate of blood that is filtered by glomeruli in each minute (2). The GFR is the best indicator of CKD level determination; so that a decrease in GFR shows a decrease in kidney function (3). Generally, CKD is defined through either kidney damage with/without decreased GFR, or decreased kidney function (GFR<60 mL/min/1.73 m^2) with/without kidney damage (each of these situations may last 3 months or more). According to the Kidney Disease Improving Global Outcomes (KDIGO), the staging subjects who have GFR in classes of ≥90, 60-89, 30-59, 15-29, and <15 are in the stages 1 to 5 of CKD, respectively (4). The kidney damage occurs in the advanced stages of the disease which cannot be cured (5). Furthermore, the risk of death from cardiovascular and blood vessel diseases will be increased due to the CKD progress (6,7). In this condition, patients need invasive and costly treatments such as dialysis or kidney transplant to survive (8). The CKD has been recognized as a serious public health threat (9). Globally, the burden of CKD has been rapidly rising. In addition, the incidence and prevalence of CKD are dramatically increasing (10). The range of global prevalence was estimated 8% to 16% and the pooled incidence was 25.8/100 person/year (11,12). The place of death due to CKD had been changed from 27th to 18th place in 1990-2010 between all deaths (13). Furthermore, death due to CKD was estimated about five to ten million people,

based on the GBD study in 2015 (14). In Asian countries, the mean of CKD prevalence was almost 10% (15). In Iran in 2004, the studies estimated the CKD prevalence and incidence rates to be 1083 and 173.5 per 100,000 population. The Disability Adjusted Life Years (DALY) of CKD in the stages 1 to 4 was obtained 1124164 in 2004, which reflects a high burden of the disease in the last decade (16). The early detection of CKD is a useful way to reduce the disease burden. In addition, it can prevent progression and adverse outcome of the disease (17).

In order to do early detection, assessing the kidney damage and estimating the GFR (eGFR) is recommended and it can be measured by inulin urinary clearance (18). However, measuring clearance is too costly and is not used as a routine clinical application (19). In contrast, measuring creatinine of blood, which determines the GFR, is a common method; this method is affected by the muscle mass, gender, and age (20). Therefore, creatinine has limitations, which make it inappropriate to determine GFR and predict CKD. The studies have shown that cystatin C (Cys-C) is another marker for predicting the early stages of the disease (21). Cys-Cis is a small protein (13 kDa) that belongs to the Cys-C protease family. This marker is produced in all nucleated cells and the production rate is constant. Unlike the creatinine, the level of Cys-Cis is not affected by the presence of inflammatory conditions, muscle mass, gender, body conditions, and age (after 12 month) (22). Neutrophil gelatinase-associated lipocalin (NGAL) is a biomarker introduced by the researchers to detect the early stage of kidney damage. NGAL is a 25-kDa protein which belongs to the lipocalin protein family and can be found simply in the blood and urine. This small molecule is not a marker of renal function, but it has been recognized as a promising marker for the renal injury (23,24). NGAL was introduced as one of the best markers for the early detection of CKD, and acute kidney injury (AKI). In addition, it is a significant prognostic factor in detecting acute myocardial infarction and heart failure (23,25,26).

Some studies have been done to examine the correlations between variables Cys-C and NGAL, and eGFR and showed significant correlation between them (27,28). However, we did not find a study that examined the predictive roles of each marker alone. In the present study, we aimed at assessing the Prediction Power of Cys-C, NGAL, and combination of them for the early detection of CKD using logistic regression model.

Materials and Methods

Participants and procedure

This cross-sectional study included 72 patients (48 women and 24 men) aged between 40 to 70 years old. They were selected among renal patients who referred to the Shohadaye Tajrish Hospital to check their kidney function between 2012 and 2103. The selected volunteers had no chronic illness such as cardiovascular and liver diseases. Furthermore, the patients with the stable renal disorder had their creatinine and urine in normal levels (29).

At the beginning of the study, the volunteers' blood samples were collected in order to measure biochemical parameters. The samples were stored at -20°C until the plasma NGAL and Cys-C were evaluated by enzyme-linked immunosorbent assay (ELISA) method. The eGFR was assumed as the gold standard method in which the fixed value 78 mL/min/1.73 m^2 was chosen as a cut-off between patients with and without CKD. In this study, the NGAL and Cys-C were used as predictors and eGFR was used as a response variable. This study was approved by the Medical Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RET ECH.REC.1397.1013).

Statistical Analysis:

The statistical analysis was performed using the IBM SPSS 25.0 (IBM Corp., Armonk, NY, USA). For univariate purposes, the Chi-square and independent samples *t*-tests were utilized. In addition, to investigate the predictive power of the biomarkers (NGAL and Cys-C), the univariate and multiple logistic regression model and receiver operating characteristics (ROC) curve analysis were used. In this process, the following logistic regression models were applied in which the binary outcome was eGFR (with the cut-off 78 mL/min/1.73 m^2).

(1) Model with only "NGAL" as the predictor

(2) Model with only "Cys-C" as the predictor

(3) Model with both "NGAL" and "Cys-C" as the predictors

In all of these models, the predicted probability of having CKD ($\Pi_i(x)$) for each person was calculated using the general formula $\pi(x) = \exp(\alpha + X`\beta) / (1 + \exp(\alpha + X`\beta))$, Where α is the intercept, and β is vector of regression parameters. The ROC curve and the Youden Index were used to evaluate the diagnostic power of markers and determine the optimal cut-off points. This cut-off point was used to compare the predicted probabilities and classify each individual as patient or non-patient. According to the obtained cut-off points, then, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under ROC curve (AUC) were calculated.

Results

The total number of 72 patients who referred to the Shohadaye Tajrish Hospital for checking their renal function were studied. In this sample, 66.7% (48 patients) were female and 34.7% (25 patients) had kidney damage. The Mean (SD) age and weight for the entire sample were 54.51 (8.06) and 79.28 (13.65) years, respectively. The mean (SD) NGAL and Cys-C levels of the sample were 78.72 (105.44) ng/mL and 423.72 (744.96) ng/mL, respectively.

Table 1 shows the results of logistic regression models which were described in the methods section. In the firs model, (model with only NGAL as the predictor), one can observe that one unit increase in NGAL value can raise the odds of having renal damage by 14.2% (*P=0.001*). For the model with only Cys-C as the predictor, it can be seen that one unit increase in Cys-C value has only 0.4% increase in odds of CKD resulted (*P=0.002*). Finally, in the third model (model with both NGAL and Cys-C as the predictors), only NGAL showed a significant relationship with the kidney damage (*P=0.007*). According to this model results, one unit increase in NGAL value could lead to about 12.5% increase in odds of kidney damage.

Table 2 summarizes the findings from ROC curve analysis for the three fitted models in Table 1. Moreover, ROC curve for each model was shown in Figure 1. Regarding these findings, one can conclude that the model with only NGAL as the predictor could result in a rather perfect prediction of renal disorder.

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Table 1. Results of logistic regression model for determining the power of NGAL and Cys-C in predicting kidney damage

Model	Variable	Coefficient	Standard Error	Odds Ratio	95% Confidence Interval	P-value
Model1	NGAL	0.133	0.041	1.142	1.045-1.237	0.001
Model2	Cystatin C	0.003	0.001	1.004	1.001-1.006	0.002
Model3	NGAL	0.118	0.044	1.125	1.032-1.226	0.007
	Cystatin	0.001	0.001	1.001	0.999-1.003	0.505

Table 2. Results of ROC analysis

Model	Variables	Sen* (%)	Spe** (%)	PPV (%)	NPV (%)	AUC	Cut Point
1	NGAL	96.00	100.00	100.00	97.92	0.992	0.69
2	Cys-C	64.00	97.87	94.12	83.64	.851	0.28
3	Cys-C & NGAL	96.00	100	100	97.92	0.992	0.63

***sensitivity** ****specificity**

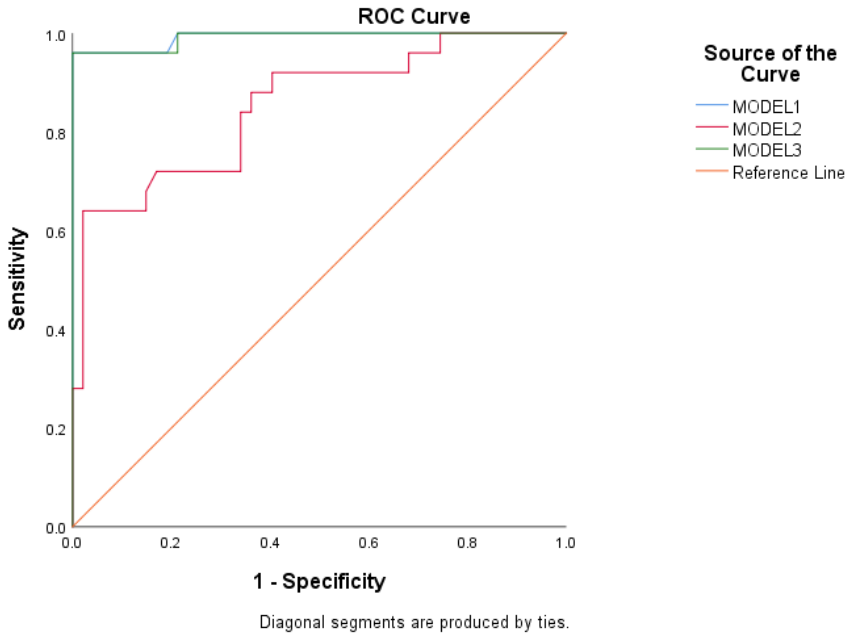

Figure 1. ROC curve for model1, model2 and model 3

Discussion

In this study, we used simple and multiple logistic regression models for assessing the power of Cys-C and NGAL for the early prediction of CKD. Our findings showed that the model with NGAL as the single predictor had rather perfect predictive power (all of the diagnostic accuracy measures were close to 1.0). In addition, the Cys-C biomarker revealed no significant relationship with CKD. In other words, adding Cys-C to a model, which includes the NGAL biomarker, did not lead to increase in the value of diagnostic accuracy measures.

Cys-C is a biomarker of CKD, which is produced in all nucleated cells. It is not affected by the muscle mass, gender and age **(21)**. In this study, in order to evaluate the prediction power of Cys-C biomarker, the cut-off point 78 mL/min/1.73 m^2 was considered for eGFR. Accordingly, the model which only included Cys-C had low sensitivity, acceptable specificity and AUC 64%, 97.87% and 85%, respectively. However, in a study on 206 patients in Sweden, White *et al.* aimed to assess the diagnostic accuracy of Cys-C biomarker. The cut-off points of their study were 72 ml/min/1.73 m^2 and 1.25 mg/L for eGFR and Cys-C, respectively (30). They found greater sensitivity and less specificity for Cys-C (71.4% and 95.1%, respectively). In addition, Martin *et al.* assessed 94 patients for evaluating the diagnostic accuracy of Cys-C in advanced stages of CKD. They had CKD as a result of different diseases such as diabetes **(31)**. Using the analysis of ROC curve, they showed that in sever stages of CKD, Cys-C had AUC of 63%, while, in our study which was performed on the early stage of CKD patients, this value was 85%. According to this, it can be concluded that Cys-C may have more diagnostic accuracy in the early stage of the disease.

NGAL is a promising marker for the kidney damage, which can be easily found in blood and urine. The results of our study also proved high prediction power of NGAL for the early-stage renal damage in CKD patients **(32)**. The models, which include NGAL and defining optimal cut-off point for probabilities, resulted in proper sensitivity, specificity, NPV, PPV and AUC for NGAL (96%, 100%, 100%, 97.92% and 99%, respectively). However, in a prospective cohort study on patients between 18 to 65 years old Basturk *et al.* evaluated the diagnostic accuracy of NGAL biomarker. Their study showed that NGAL in its optimal cut-off point had less sensitivity and specificity (72.2%, and 72.2%, respectively) **(29)**. Also, in another study on the end stages of CKD patients, they showed that considering best cut-off point for NGAL it had less sensitivity and specificity (83.9% and 53.8%, respectively) **(33)**.

Ghonemy *et al.* considered the NGAL and Cys-C as the markers of Acute Kidney Injury after cardiac surgery. They showed, even in this condition, the sensitivity and specificity of NGAL were higher than Cys-C **(34)**. In this study, in the early stage of CKD, the model with NGAL showed the highest diagnostic measures as well. Mitsnefes *et al.* showed that NGAL had a better diagnostic performance than Cys-C for a cut-off point of eGFR <30 mL/min/1.73 m^2 and they also concluded that NGAL and Cys-C were similar at GFR levels of ≥30 mL/min/1.73 m^2 **(35)**.

None of the above-mentioned studies evaluated NGAL and Cys-C markers simultaneously in order to predict CKD; while the logistic model is an appropriate model for binomial data, none of these studies used it for evaluating the kidney damage. In the field of CKD prediction, Fisher *et al.* used logistic regression model including 12 covariates such as age, race, hypertension, etc. They performed their study on 11955 adults over 18 years old **(36)**. The results of their study, comparing to our results, showed less sensitivity and specificity (86% and 85%, respectively).

Despite some advantages such as concurrent analysis of biomarkers, our study had some limitations. First, this study had a small sample size and generalization of the results may be questionable. Second, as measuring GFR is costly and time-consuming, we used the eGFR as the gold standard for CKD. As we know, eGFR is an estimate of GFR, thus some imprecise values may be included in the data set. In order to solve this problem, we aimed to assess NGAL and Cys-C markers using a Bayesian approach without considering gold standard in our future study.

Conclusion

The findings revealed that the NGAL biomarker resulted in satisfactory power for predicting the early kidney damage. In addition, we found that the predicting power of Cys-C for early diagnosis of kidney damage is less than NGAL biomarker. Further studies with higher sample size are needed to evaluate the prediction role of NGAL and Cys-C using other methods such as machine-learning techniques, support vector machines (SVM) and decision tree analysis. Moreover, we suggest studies

with longer follow-up to know the proportion of patients with renal damage.

Conflict of Interest

The authors declared no conflicts of interest.

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