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Original Research Article

Case Control Study to Predict the Diagnostic Importance of Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a Biomarker for Chronic Kidney Diseases.

Amita Gupta[1], Rajeev Lohokare[2], Purnima Dey Sarkar[3], Akshatha R[4]

[1]Assistant Professor, Department of Biochemistry, Mahatma Gandhi Memorial Medical College, Indore, MP, India.

[2]Associate Professor, Department of Biochemistry, Mahatma Gandhi Memorial Medical College, Indore, MP, India.

[3]Professor, Department of Biochemistry, Mahatma Gandhi Memorial Medical College, Indore, MP, India.

[4]PG resident, Department of Biochemistry, Mahatma Gandhi Memorial Medical College, Indore, MP, India.

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Corresponding author: Dr. Amita Gupta

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Abstract

Introduction: The incidence of kidney disease is reaching to epidemic proportions. Early intervention can significantly improve the prognosis of kidney diseases. However, the paucity of early, predictive, non-invasive biomarkers has impaired the timely effective managements in these cases. In our study a new biomarker neutrophil gelatinase-associated lipocalin (NGAL), is investigated for its role in chronic kidney disease (CKD).

Aim: To assess the role of Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a potential biomarker of Chronic Kidney Disease (CKD) in comparison to serum urea, serum creatinine and Creatinine Clearance Rate (CCR).

Materials and Methods: This case-control study was conducted at MGM Medical College, Indore, MP, India, from October 2022 to March 2023. Total 50 known patients of kidney diseases and 50 healthy individuals above the age of 18 years were enrolled in the study. Blood samples were collected from all individuals and serum NGAL, serum urea level, serum creatinine level, fasting blood sugar were measured. Correlation of NGAL with serum urea level, serum creatinine level, CCR was calculated by Pearson Correlation test.

Results: In present study, 50 patients in case groups (28 male and 22 females) and 50 healthy controls (26 males and 24 females) were included. Among controls, the mean age of patients was 53.14±5.62 years and among cases 52.74±8.56 years. NGAL level was increased two times (from 106.28±43.72 ng/mL to 296.17±62.65 ng/mL) in CKD patients than controls individuals. NGAL level was positively correlated with serum urea level, serum creatinine level while negatively correlated with CCR.

Conclusion: The NGAL may be a useful and reliable serum marker for identifying the magnitude of renal dysfunction in patients with CKD and may have its place beside serum creatinine as an alternative endogenous GFR marker.

Keywords: Chronic Kidney Diseases, NGAL, Urea, Creatinine, Biomarker.

Introduction

Chronic kidney disease (CKD) evolves over many years, is a major public health problem . The prevalence of all stages of CKD varies between 7% and 12% in the different regions of the world.[1] Serum creatinine is commonly used as a biomarker of renal function, high serum creatinine is primarily a marker of glomerular filtration [2] and cannot be considered as an ideal for the estimation of kidney injury, because it is influenced by muscle mass, race, gender, and medications and not reliable for the diagnosis of renal tubular injury in the absence of significant reduction in the glomerular filtration rate (GFR) [3]. Liver produces creatinine and is immediately affected by hepatic parenchymal dysfunction, it cannot accurately reflect GFR in a number of disorders [4].

For screening of the severe kidney diseases earlier and more accurately, still glomerular filtration rate (GFR) is the ideal marker. Unfortunately, measuring GFR is time-consuming and therefore GFR is usually estimated from equations that take into account endogenous filtration markers, such as

serum creatinine (SCr) and cystatin C (CysC). [5,6] Another important biomarker, albuminuria, [7, 8] precedes kidney function derangement and has been demonstrated as having strong associations with disease progression and outcomes.

Presence of CKD is confirmed when abnormalities in kidney function tests, like abnormal GFR, albuminuria or proteinuria occurs for a minimum of three months . 5 stages of chronic kidney disease are known, starting with mild kidney impairment and progressing to complete kidney failure. The prognosis and course of therapy for CKD are variables. Treatment aims to slow down the progression of the disease, reduce its symptoms, and prevent further complications of the disease. Treatment for CKD can involve lifestyle changes like diet and exercise, blood pressure and glucose monitoring and medical interventions including drugs to lower proteinuria and complications. The efficacy of treatment was conditional on the nature and degree of the patient's condition as well as their willingness to take medications . Early diagnosis and promt treatment of CKD can halt the progression of the disease and improve patient outcomes. Many people with end-stage renal disease need dialysis or require kidney transplant for sustainability. Therefore, CKD outcomes and quality of life can be improved by early diagnosis and treatment [9,10].

CKD is known to be related with diabetes, obesity, hypertension, aging, and other cardiovascular diseases; the presumptive pathological findings are diabetic glomerulosclerosis and hypertensive nephrosclerosis [11]. Diabetes mellitus and hypertension are the two main leading causes for end-stage renal disease (ESRD) characterized by reduced renal function and/or albuminuria [12]. Progression of kidney diseases can be prevented by early detection and treatment as established over past twenty years. [13]. A biomarker which is able to detect early kidney damage as well as to identify patients at an increased risk of progressive disease would impact kidney disease diagnosis and treatment. Neutrophil Gelatinase-Associated Lipocalin (NGAL) has been proposed to be useful for the diagnosis of CKD [14] and it has the potential to be an ideal biomarker in the early detection of CKD. Goetz et al in their study has described the main features of NGAL.[15,16]

Neutrophil gelatinase–associated lipocalin (NGAL; also known as human neutrophil lipocalin, lipocalin-2, siderocalin, 24p3, or *LCN2*) is a small molecule of almost 25 kd lipocalin iron-carrying protein that belongs to the well-defined superfamily of proteins called lipocalins. The lipocalins share a molecular organization comprising 8-strands arranged in a complex-barrel structure delineating a calyx shape, which represents their binding site. [17]

Neutrophil gelatinase-associated lipocalin (NGAL), was first originally isolated from neutrophils. [18] Then people found that it also expressed in tissues such as kidney, liver, epithelial cells [19, 20] and vascular cells in atherosclerotic plaques.

Recent study aim to define whether NGAL have a certain role in renal and systemic changes in patients of chronic kidney diseases . The principal hypothesis was that chronic renal damage could influence the physiological balance of this protein in a way similar to that observed for acute injury conditions. In this view, chronically damaged tubular cells would produce a great quantity of NGAL.

Materials & Methods:

This case-control study was conducted at MGM Medical College, Indore, MP, India, from October 2022 to April 2023 after approval from the Institutional Ethics Committee.

Inclusion Criteria: Patients of more than18 year age with established diagnosis of CKD. Apparently Healthy volunteers of age more than18 years were taken as control.

Exclusion Criteria: Subjects with primary tubular diseases, on medication with potentially nephrotoxic drugs, acute kidney injury, terminal kidney failure requiring dialysis were excluded from the study.

Fifty patients of known CKD were selected as cases from MY Hospital, Indore randomly. Fifty healthy individuals working in MGMMC were selected as controls.

Prior enrolment for this study an informed written consent was obtained from all the participants. Only consenting participants were included in the study. All enrolled participants were subjected to demographic, anthropometric and biochemical analysis following the standard protocols. The detailed history with full clinical examination were taken in all the cases.

Sample Collection

Under aseptic precautions, 5 mL of venous blood sample was drawn after an overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000 rpm for 20 minutes for separation of serum. Serum was divided into two parts in aliquotes at -20°C. One part was used for the estimation of NAGL. The other part of the serum was used for estimation of creatinine and urea level.

Serum sample was analysed for creatinine and urea level by the auto analyser RANDOX Automated Analyzer based on the principle of sphectroph-otometry.

Serum NAGL was estimated by ELISA method using commercially available kits. Detection range: 31.25-2000 pg/mL and Sensitivity: 18.75 pg/mL of the kit.

Determination of CCR: Creatinine clearance was estimated by using serum creatinine levels. The Cockcroft-Gault (C-G) formula 16 uses a patient's weight (kg) and gender to predict CrCl (mg/dL). The resulting CrCl is multiplied by 0.85 if the patient is female to correct for the lower CrCl in females. The C-G formula is dependent on age as its main predictor for CrCl. Below is the formula:

eCCr=(140-Age) × Mass (kg)×(0.85 if female)/ 72×{Serum Creatinine (mg/dL)} [21].

Statistical Analysis

The statistical analysis was done by using the IBM SPSS Statistics software version 20.0 for Windows. Continuous variables were presented as mean ± standard deviation (SD). An independent t-test was employed to compare the parameters between the case and control groups. The correlation between the parameters was assessed using the Karl Pearson's coefficient test. . *p*-value of less than 0.01 considered as statistically significant.

Results

Table 1: Demograp hic data of the cases/controls involved in the study.

Variables	Mean ± SD						P- value
	Controls			Cases			
	Total	Male	Female	Total	Male	Female	
Gender	50	26	24	50	28	22	0.52
Age (years)	53.14±4.4	55.23±6.2	51.21±2.1	55.68±3.2	54.96±1.2	52.78±3.3	**0.037**
Height(mt)	1.62 ±0.03	1.65±0.09	1.61±0.08	1.66±0.05	1.65±0.03	1.62±0.04	**0.043**
Weight(kg)	66.3±10.3	69.45±9.4	68.2±7.6	67.4±8.9	70.5±10.4	65.7±8.6	0.12
BMI(kg/m^2)	26.35±1.2	27.38±1.8	28.87±1.9	28.56±1.3	25.86±1.4	27.3±1.6	**0.05**

Chi-square test, independent t-test; BMI: Body mass index; **Bold p-values are significant.**

Table 2: Comparison of urea, creatinine, creatinine clearance rate, serum NGAL levels between the CKD and non-CKD subjects by independent t-test

Variables	Case	Control	t value	p value
	Mean ± S.D.	Mean ± S.D.		
Urea(mg/dl)	98.72±43.20	29.45±5.16	15.87	**<0.001****
Creatinine(mg/dl)	3.62±0.38	0.81±0.12	26.67	**<0.001****
CCR	36.30±10.68	94.76±9.80	-42.38	**<0.001****
Serum NGAL(ng/ml)	296.17±62.65	106.28±43.72	21.40	**<0.001****

**Correlation is significant at the 0.05 level

Table 3: Pearson correlation of NGAL with creatinine, urea and creatinine clearance rate (CCR)

Analysis	Creatinine	Urea	CCR
Pearson correlation	0.98	0.98	-0.97
p-value	**<0.001**	**<0.001**	**<0.001**

Correlation is highly significant at 0.01 level; **bold p-values are significant**

Discussion

Researchers have developed a variety of indicators for assessing kidney function. The glomerular filtration rate (GFR) was known as the best indicator of renal function among the existing biomarkers . Other renal functions and biomarkers in CKD patients also altered along with GFR [22,23].

In this case-control research, the blood NGAL levels of 50 CKD patients and 50 non-CKD participants were examined .The present study found that serum NGAL levels were significantly higher in CKD subjects compared to non-CKD subjects (P<0.001). This study also demonstrated a significant positive correlation between NGAL, urea, and creatinine (P<0.05). NGAL levels also exhibited a significant negative correlation with creatinine clearance rate. Similar results have been reported in multiple studies, indicating that NGAL is a highly sensitive biomarker with lower specificity for CKD. So that NGAL could be utilized as a single reliable biomarker for early detection of CKD [24-26]. On the other hand, some studies reported contradicting results compared to our study and they did not observed any significant associations of NGAL with CKD [27,28].

There was evidence that NGAL may also be used as a mediator of CKD progression; Previously, NGAL was identified to be one of the first highly active genes and proteins in tubular epithelial cells in the distal nephron, and that it was released from these cells following tissue damage, such as ischemic renal injury.

Results of the present study showed that in patients of CKD, serum level of urea and creatinine was elevated by approximately three times. However, the elevation in serum urea level and serum creatinine level was 210.15% and179.21%, respectively in males while in female groups this increment was found to be higher (224.64% and 213.35%, respectively). Thus, the elevation was higher in female patients than male. CCR was lowered in patients with renal impairment than control. These findings were in accordance to some previous findings which have concluded that the increased serum creatinine has a renal cause and consider a result of reduced CCR which is also related to increased serum urea concentration [29,30].

Findings from the present study clearly indicate that NGAL represents a novel risk marker of CKD progression.

Conclusion

Present study shows a positive correlation of the NGAL levels with those of creatinine and urea ,it is inversely correlated with creatinine clearance rate in the patients of CKD.NGAL may be a useful biomarker for the prediction of kidney injury as well as it might can help in the early diagnosis and intervention in the patient suffering from CKD.

Limitation of Study

As it is a case control study with limited sample size at a single centre may limit generalizability of the results. In future more such studies are needed to establish the role of NGAL in CKD.

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