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Original Research Paper

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STUDY OF SERUM NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN CONCENTERATIONS AS A MARKER OF RENAL FUNCTION IN CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT

Introduction: Serum lipocalin 2 serve as a marker for kidney function. Lipocalin 2 is found in both CKD and kidney injury and it rises in acute kidney injury (AKI) and in patients have faster decline in kidney function. **Aims And Objectives:** To find out correlation and assess of serum Neutrophil gelatinase-associated lipocalin 2 (NGAL 2) in patients with stages 2 to 4 of Chronic Kidney disease. The aim of the study was NGAL could represent a novel, sensitive marker of kidney function in adult patients with CKD. **Material And Methods:** Study involved 120 patients divided in Case group (60 patients) attended medical/ urology OPD or admitted in medical/urology ward of CKD2 – CKD4 while control group – age and sex matched healthy individuals/ stage I CKD patients was taken as control. The plasma/ serum were used for serum urea, creatinine, Cystatin C and lipocalin 2 under all aseptic precaution on receiving consent. **Result:**The patients of CKD included in study were having glomerulonephritis (46.7%), pyelonephritis (21.7%), diabetic kidney disease (13.3%), polycystic kidney disease (1.7%) and other causes (16.7%). CKD patients demonstrated elevated serum NGAL 159.14 ± 48.73 ng/ml, together with a rise in urea 59.9 ± 17.6 mg/dL, serum creatinine 1.56 ± 0.97 mg/dL and Cystatin C 199 ± 113 ng/ml as compared to control have serum NGAL 76.31 ± 26.34 ng/ml, urea 22.3 ± 5.7 mg/dL, serum creatinine 0.75 ± 0.14 mg/dL and Cystatin C 76 ± 17 ng/ml (P value <0.05). **Conclusion:** Serum NGAL closely correlates with serum Cystatin C, creatinine, and eGFR, and serve as a potential early and sensitive marker of impaired kidney function/ kidney injury.

KEYWORDS : Neutrophil Gelatinase Associated Lipocalin, Chronic Kidney Disease, Glomerular Filtration Rate, Acute Kidney Injury.

INTRODUCTION:

Chronic Kidney Disease was defined by the reduction of glomerular filtration rate (GFR) to less than 60 mL/min/1.73 m^2 and/or evidence of kidney damage, such as proteinuria (albuminuria > 30 mg/g of creatinine), glomerular-based or tubular-based hematuria (not urologic), or abnormal renal imaging and pathologic abnormalities of 3 months duration or longer, irrespective of the cause. According to International Society of Nephrology's Kidney Disease Data Center Study recent report, the prevalence of Chronic Kidney Disease is 17%. In India different regions have prevalence ranges from < 1% to 13%. [3] Non communicable diseases such as chronic kidney disease are emerging as an important public health problem with its high prevalence, morbidity and mortality. Chronic Kidney Disease (CKD) is found to be one of the causes of mortality in Indian subcontinent. [1,2] However GFR is used to determine the stage of kidney disease and its function. Glomerular filtration rate tells about the kidney functions. There are 5 stages in Chronic Kidney Disease which are based on Glomerular filtration rate.

Table1: - KDIGO classification of CKD

S. No.	GFR Stages	GFR (mL/min/1.73 m^2)	Terms
1.	G1	>90	Normal
2.	G2	60–89	Mildly decreased
3.	G3a	45–59	Mild to moderately decreased
4.	G3b	30–44	Moderate to severely decreased
5.	G4	15–29	Severely decreased
6.	G5	<15	Kidney failure

The Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines advocate that serum creatinine is a very sensitive marker for estimating GFR in patients with potential kidney disease and to classify in stage of disease. [4]

Neutrophil gelatinase-associated lipocalin (NGAL), barrel like structure protein (secreted or cytoslic) carry fatty acid, retinoids, pheromones etc as hydrophobic ligand, belong to lipocalin superfamily. Expression of NGAL mRNA occurs rapidly in case of acute renal injury. Serum NGAL might provide an additional accurate measure of kidney impairment in CKD, particularly at advanced stages. [5]

Various studies suggest that both parameters are expressed by tubular interstitial damage and atrophy in course of chronic kidney disease. Studies suggested that lipocalin level raises in acute kidney injury (AKI) and patients who are at higher risk of faster decline in kidney function. Various studies of lipocalin show positive as well as negative correlation with GFR, creatinine and Cystatin C. Reliability; sensitivity and high diagnostic accuracy of both parameters between healthy individuals and CKD patients were assessed.

MATERIAL & METHODS:

The study was conducted on two age and sex matched group of participants attending medical OPD/ urology OPD and Biochemistry Lab of Jawahar Lal Nehru Medical College and Hospital, Ajmer, Rajasthan. The study was conducted in total 120 patients divided in two groups of 60 each. Case group was consist of 60 patients attending medical OPD/urology OPD or admitted in medical/urology ward of CKD 2 – CKD 4 while control group was consist of 60 age and sex matched healthy individuals/ stage I CKD patients were taken as control. Informed and written consent were taken from all the participants. This study was reviewed by the ethical committee.

Inclusion criteria

1. Chronic Kidney disease stage 1 to stage 4
2. Glomerular filtration rate < 90
3. Age > 18 years

Exclusion criteria

1. Pregnancy or lactation
2. Patients with End Stage Renal Disease, dialysis and cardiac conditions
3. Patients on treatment with immunosuppressive drugs
4. Age < 18 years

Blood samples were collected in plain vial for estimation of urea, creatinine, Cystatin C, uromodulin and lipocalin 2 by venipuncture, under all aseptic precaution on admission. The samples were collected and serum was stored at – 70° C. The measurement of serum urea by Berthelot enzymatic colorimetric method [6], Serum Creatinine by Jaffe's colorimetric kinetic method [7], Serum Cystatin-C by Enzyme Linked Immunosorbent Assay (Cystatin C Human ELISA Kit, Invitrogen, Thermo Fisher scientific), Serum Lipocalin 2 by Enzyme Linked Immunosorbent Assay (Human Lipocalin 2 ELISA Kit, Invitrogen, Thermo Fisher scientific) was done.

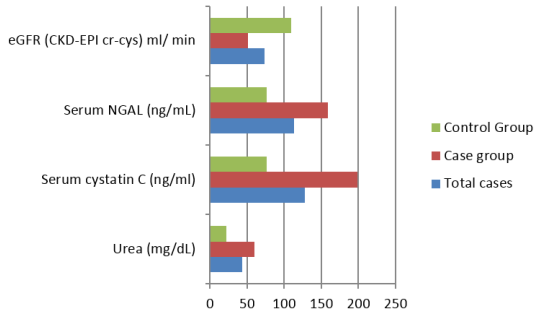
RESULT:

The patients of CKD included in study were having glomerulonephritis in 46.7% of case, pyelonephritis in 21.7% of cases, diabetic kidney disease in 13.3% of cases, polycystic kidney disease in 1.7% of cases and other causes were found in 16.7% of cases.

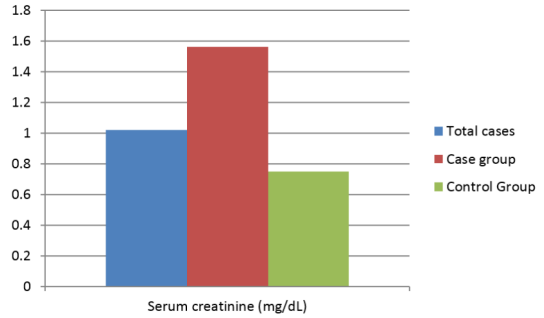
CKD patients (case group) demonstrated elevated serum NGAL level 159.14 ± 48.73 ng/ml, together with a rise in urea 59.9 ± 17.6 mg/dL, serum creatinine 1.56 ± 0.97 mg/dL and Cystatin C 199 ± 113 ng/ml level as compared to control group have serum NGAL 76.31 ± 26.34 ng/ml, urea 22.3 ± 5.7 mg/dL, serum creatinine 0.75 ± 0.14 mg/dL and Cystatin C 76 ± 17 ng/ml (P value <0.05).

Table 2 – Characteristics of the study group

Parameter	Total cases (120)	Case group (60)	Control Group (60)
Age (years)	45.68 ± 11.08	43.32 ± 12.86	44.18 ± 16.96
BMI (kg/m^2)	25.9 (22.7–29.3)	24.9 (22.1–29.4)	27.5 (23.9–30.9)
Systolic blood pressure (mmHg)	133 ±15	131 ±12	132 ±16
Diastolic blood pressure (mmHg)	81 ±7	79 ±8	83 ±10
Urea (mg/dL)	43.2 ± 11.8	59.9 ± 17.6	22.3 ± 5.7
Serum creatinine (mg/dL)	1.02 ± 0.16	1.56 ± 0.97	0.75 ± 0.14
Serum cystatin C (ng/ml)	128 ± 49	199 ± 113	76 ± 17
Serum NGAL (ng/mL)	113.35 ± 38.05	159.14 ± 48.73	76.31 ± 26.34
eGFR (CKD-EPI cr-cys) ml/ min	73.57 ± 7.93	51.22 ± 8.09	109.13 ± 19.09



Graph 1 – Indicating correlation of eGFR, Urea, Cystatin C and NGAL



Graph 2 – Indicating correlation of Serum Creatinine with case, control and total patient group

DISCUSSION:

In our study elevated serum NGAL level was found associated with serum creatinine and cystatin C level in CKD patients. Therefore serum NGAL is closely related with serum creatinine, cystatin C and GFR so can serve as a potential marker in patients with impaired kidney function. NGAL is belonging to protein compound which falls in lipocalin family. It was originally isolated from activated neutrophils (human) and found in low level in kidney. [8] it is synthesized in kidney injury which is finally followed by glomerular filtration and uptake by injured tubules systemically. Bolignano et al has found a close correlation between serum NGAL levels with residual renal function found in 26 APKD patients. [9]As in our study Levey et al [10]also found that NGAL can be used as marker of renal function in children.

CONCLUSION

CKD patients demonstrated elevated serum Lipocalin 2/ NGAL together with a rise in serum creatinine and Cystatin C. Moreover, Lipocalin 2/ NGAL closely correlated with serum Cystatin C, creatinine, and eGFR, and could serve as a potential early and sensitive marker of impaired kidney function/ kidney injury.

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