

How to Cite:

Abdulameer, A. N., & Jaffat, H. S. (2022). Physiological study of NGAL and Cystatin C in chronic and acute kidney disease patients. *International Journal of Health Sciences*, 6(S3), 8329–8343. <https://doi.org/10.53730/ijhs.v6nS3.7900>

Physiological study of NGAL and Cystatin C in chronic and acute kidney disease patients

Ali Nahedh Abdulameer

University of Kufa, Faculty of Science, Department of Biology, Iraq

Email: ali95alruazik@gmail.com

Haider Salih Jaffat

University of Kufa, Faculty of Science, Department of Biology, Iraq

Email: hayder.alshafie@uokufa.edu.iq

Abstract---Kidney disease is an important global health concern, affecting ~15% of the global population and typically associated with systemic disorders (e.g., diabetes). Increased incidence of kidney diseases and their progression to critical stages are facing many nations across the globe. The current study was performed with 65 male patients (20 with Acute kidney disease and 45 with chronic kidney disease, their mean age (44.38±14.59) years, who have visited the physician's consultancies and the kidney disease unit at Al-Sadder Teaching hospital in Al-najaf, Iraq, and 25 persons as a healthy control group their mean age (48.68±16.37) years, They were all Males with no prior medical history and no indications or symptoms of renal disease, diabetes, anemia, hypertension, thyroid illness, dyslipidemia, or obesity . The study was conducted from September 2021 to May 2022. Biomarker appropriate for kidney failure monitoring should have narrow biological variability Such as NGAL and Cystatin C Were biomarkers of tubular epithelial cells injury and pathogenesis of KD progression, it was predicted to be reflecting disease activity and kidney function. In this study, serum NGAL and Cystatin C levels were measured in patients and healthy groups. It also assays the relationship between levels of these biomarkers with some biochemical parameters PTH, Creatinine, Urea, GFR, Calcium, And Phosphor. The results of our study indicated there was significant difference (p-value<0.05) decreased with mean± SD of eGFR in CKD and AKI patients (13.42±13.83 ml/min/1.73) as compared with healthy control group (114.12±12.49 ml/min/1.73 m2). The results also indicated that there is significant difference (p-value<0.05) increased in the mean level of urea (156.6±53.97mg/dl), creatinine(8.57±4.25mg/dl), phosphorus (7.99±4.03 mg/dl) and blood sugar (177.92±74.11), in patients with CKD and AKI as compared with healthy control group (26.76±6.26 mg/dl) (0.7±0.12 mg/dl),(

3.49±0.52 mg/dl), (93.99±10.75), respectively. There is also significance difference (p-value<0.05) decreased levels in the mean value of calcium in patients with Kidney failure (6.82±1.41 mg/dl) as compared with healthy control group (9.17±0.56 mg/dl). There was no significance difference (P>0.05) in the mean levels of NGAL and cystatin C in Chronic kidney disease patients as compared with acute kidney disease patients. In conclusion, serum NGAL and Cystatin C levels are significant biomarkers for predicting progressive kidney disease irrespective of recognized risk factors. As a result, these biomarkers are an effective tool for investigating patients at high risk of GFR reduction and may serve as an enrichment marker for clinical groups aimed at preventing CKD development.

Keywords---NGAL, Cystatin C, chronic, acute kidney, disease patients.

Introduction

Kidney disease is an important global health concern, affecting ~15% of the global population and typically associated with systemic disorders (e.g., diabetes). Increased incidence of kidney diseases and their progression to critical stages are facing many nations across the globe (De silva *et al.*, 2021). It is increasingly obvious that repeated AKI can lead to the development of chronic kidney disease (CKD) and then end-stage renal disease (ESRD) through a progressive decrease in renal function without obvious symptoms in the early stages (Rysz *et al.*, 2017), especially in high prevalence communities with CKDu. a single biomarker may not provide an early diagnosis considering the complex pathophysiology associated with CKD. Multiple biomarkers may contribute to better diagnosis, prediction of renal outcomes (Gunasekara *et al.*, 2020). estimated glomerular filtration rate (eGFR), Serum creatinine (SCR), and the albumin creatinine ratio (ACR) are commonly used standard renal biomarkers. Current clinical definitions for acute kidney injury (AKI) rely on urine output and serum creatinine SCr (Goyal *et al.*, 2017) while definitions of chronic kidney disease rely on ACR and eGFR (Isakova *et al.*, 2017). The United States Food and Drug administration (FDA-USA) has qualified several biomarkers including Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), N-acetyl- beta-Dglucosaminidase (NAG), clusterin (CLU), Cystatin_C (Cys_C), and osteopontin (OPN) as safety biomarker to assist in the detection of renal tubular injury in phase I trials (DeSilva *et al.*, 2021).

Acute kidney injury (AKI) describes a sudden loss of kidney function that is determined on the basis of increased serum creatinine levels (a marker of kidney excretory function) and reduced urinary output (oliguria) (a quantitative marker of urine production) and is limited to a duration of 7 days. AKI is part of a variety of functional kidney conditions, which are summarized as acute kidney disease and disorders (AKD) and can range from mild and self-limiting to severe and persistent. AKD can occur without ever meeting the criterion of rapid onset of AKI, for example when kidney dysfunction evolves slowly or AKD can continue after an AKI event has ended, for example, when kidney dysfunction does not

resolve or when structural damage to the kidney persists. (Kellum et al., 2021). Chronic kidney disease (CKD) is a serious and common disease, and it eventuates in multiple complications, including premature mortality and end-stage kidney disease (ESKD). An estimated 7 to 10 adults worldwide have CKD, with only approximately 10% surviving to ESKD and only half of survivors receiving dialysis or a kidney transplant because of lack of access or high costs (Tuttle et al., 2019).

Neutrophil Gelatinase Associated-lipocalin (NGAL); is a small circulating protein that is highly modulated in a wide variety of pathological situations, making it a useful biomarker of various disease states. It is one of the best markers of acute kidney injury, as it is rapidly released after tubular damage. However, a growing body of evidence highlights an important role for NGAL beyond that of a biomarker of renal dysfunction. Indeed, numerous studies have demonstrated a role for NGAL in both cardiovascular and renal diseases. (Buonafina et al., 2018) Cystatin C a cysteine proteinase inhibitor, is freely filtered by the glomerulus and entirely reabsorbed by the proximal tubules. Contrast to SCr, the concentrations of serum cystatin C (sCysC) show a small individual variability as nonrenal factors only slightly influenced. Therefore, sCysC may be superior to SCr in detecting minimal change in the glomerular filtration rate (GFR). (Liang et al., 2020).

Aims of the study

- The study aimed to assess serum NGAL and cystatin C in patients with kidney failure and studying the correlation between these two biomarkers with some biochemical parameters Urea, Creatinine, Calcium, Phosphorus, PTH, in all subjects of this study (CKD, AKI, and Healthy group).
- Determine whether serum NGAL could be used as a biomarker for distinguishing between AKI and CKD in emergency medicine

Materials and Methods

Inclusion criteria

The current study was conducted on 65 adult subjects (20 with AKI and 45 with CKD, their mean age (44.38±14.59) years, who visited the physician's consultancy services and the kidney disease unit at Al-Sadder Teaching hospital in Al-najaf, Iraq, and 25 individuals as a control group, their mean age (48.68±16.37) years, They were healthy people with no prior medical history and no clear signs or symptoms of kidney impairment, diabetes, leukopenia, The research was carried out from Mid-2021 until May 2022.

Exclusion criteria

65 men with kidney failure were sampled, and some were found to have comorbid disorders such as cardiovascular disease, excessive hypertension, diabetes, and inherited kidney conditions. The current study excluded patients with the following illnesses: liver illnesses (including positive HBsAg and anti-HCV Ab),

systemic inflammatory (including inflammatory bowel disease), and thyroid abnormalities.

Blood collection

Collection of Blood Venous blood samples were collected between 7 a.m. and 9.00 a.m., before to the mid-week dialysis session and before heparin administration, for hematological and biochemical screening tests. Each participant and control had five milliliters of venous blood drawn. The blood was divided roughly evenly (2.5ml) and deposited in k3-EDTA tubes for the hematological profile, with the remainder (2.5ml) placed in serum tubes containing gel. These tubes were centrifuged for 5 minutes at 3000 rpm to separate the serum, which was then preserved in Eppendorf tubes. The serum was subsequently kept at -20 degrees Celsius until additional study parameters and biomarkers were evaluated.

Laboratory measurement

Measurement of biochemical parameters

- Estimation of urea
Urea was determined using the diacetyl monoxime colorimetric technique and the Berthelot reaction. An enzyme called urease converts urea to ammonia in this technique. In the presence of glutamate dehydrogenase (GDH), the ammonia generated is coupled with 2-oxoglutarate and NADH to form L-Glutamate and NAD. The reduction in NADH absorbance is related to the concentration of urea.
- Estimation of creatinine
The Jaffe reaction, a colorimetric process in which creatinine forms a yellow orange complex with picric acid in alkaline solution, was used to calculate creatinine. Photometrically, this colored complex is determined. The intensity of the generated color is related to the amount of creatinine present in the sample.
- Estimation of serum calcium
The CPC (O-cresol phthalein Complexone) techniques developed by Moorehead and Briggs enable for the determination of total calcium content in serum, plasma, or urine. CPC interacts with calcium in alkaline solution to generate a pink complex whose absorbance at 570nm is proportional to the quantity of calcium in the specimen.
- Estimation of serum phosphorus
Method without deproteinization Daly described it, and Gamst O.K. and Try K modified it. In an acidic media, phosphate ions form a phosphomolybdic combination with ammonium molybdate. The absorbance at 340 nm is proportional to the concentration of phosphate ions in the samples.
- Estimation of serum glucose
The Trinder Method. GOD oxidizes glucose to generate gluconic acid and hydrogen peroxide, which interacts with chloro4-phenol and PAP to form red quinoneimine. At 500 nm, the absorbance of the colored complex is proportional to the quantity of glucose in the material.

Estimation of NGAL and Cystatin C

The quantitative sandwich enzyme immunoassay method is used in this assay. A microplate has been precoated with a monoclonal antibody sensitive for NGAL or cystatin C. After incubation, standards and samples are pipetted into the wells, and any BIOMARKER present is collected by the coated antibody. Following thorough washing, a biotin-conjugate antibody specific for NGAL is applied to detect the captured NGAL protein in the sample. Horseradish peroxidase (HRP)-conjugated Streptavidins are then added for signal development, followed by tetramethyl-benzidine (TMB) reagent. After removing any unattached combinations, an enzyme conjugate is introduced to the wells. To cease color development, a sulfuric acid solution is applied, and the color intensity, which is related to the amount of bound protein, is detectable at 450nm.

Statistical Analysis

The analysis was carried out using SPSS v.28 and Microsoft Excel 2019 for graphics. The Kolmogorov–Smirnov test was used to assess significant deviations from normality in the distribution of continuous variables. The findings for non-normally distributed parameters were given as the median (interquartile range). The standard distribution statistical examination of group differences, and the data is presented as mean SD (standard deviations). Independent t tests are used to compare continuous variables between groups. Whenever there were numerous comparisons between groups, one-way ANOVA with Tukey post hoc was used. The chi-square test was used to examine categorical variables. Pearson's or Spearman rank was used to complete the correlation coefficient analysis. Multiple regression analyses, including binary and nominal Discrimination refers to the model's capacity to distinguish between patients and healthy controls.

Results and Discussion

Estimation of kidney functions

The results in table (1) demonstrate a significant difference (p -value <0.05) decrease in mean $SD \pm$ of eGFR in kidney failure patients (13.42 ± 13.83 ml/min/1.73 m²) when compared to the healthy control group (114.12 ± 12.49 ml/min/1.73 m²). The findings also revealed a significant difference (p -value <0.05) increased in the mean levels of creatinine (8.57 ± 4.25 mg/dl), urea (156.6 ± 53.97 mg/dl), and blood sugar (177.92 ± 74.11) in patients with kidney failure when compared to the healthy control group (0.7 ± 0.12 mg/dl), (26.76 ± 6.26 mg/dl), and (93.99 ± 10.75), respectively. These findings were consistent with many previous studies that indicated GFR and proteinuria-albuminuria are the kidney functional parameters currently used to assess KD severity (Huelin et al; 2019).

(Schaalan et al., 2016; Abhisek et al., 2016) they indicated that people with kidney failure show a significance ($p < 0.05$) increase in serum urea and creatinine when compared to healthy control group. The results also show significance difference ($p < 0.01$) decrease in GFR levels in patients with kidney failure as compared with healthy control group, GFR declines with increasing age, which is

a classic risk factor for renal failure patients. (Levey *et al.*, 2012). Reduced urea filtration may be the cause of high uric acid levels in renal failure, resulting in a reduction in (GFR) in Kidney failure patients. The measurement of urea level as an indication of renal function is based on the observation that serum/plasma urea level reflects GFR. The current investigation confirmed that the GFR level in patients fell significantly ($p < 0.01$) when compared to the healthy group, and this result agrees with previous findings. (Bobulescu *et al.*, 2012). This rise in urea and creatinine levels occurs in KF patients, because the kidney loses its ability to eliminate nitrogenous wastes from the blood, resulting in an accumulation of these substances in the blood. Creatinine is a byproduct of muscle metabolism, and an elevated level in the blood indicates kidney disease.

In general, urea buildup in the blood serum of renal failure patients results from the breakdown of meals and tissues such as muscle. Urea and creatinine levels are essential indicators because they play a key role in the diagnosis and monitoring of renal failure. Urea, a byproduct of protein metabolism, accumulates in the blood of individuals with renal failure and produces uremia (Gounden *et al.*; 2021). Calcium levels were significantly ($p < 0.05$) lower and phosphorus levels were higher in the patients' group, and this finding corresponded with those found by previous studies. (Lim *et al.*, 2014; Ozmen *et al.*, 2007). Decreased Calcium levels are observed when the kidneys fail, reducing their capacity to reabsorb calcium and resulting in calcium loss in the urine. (Worcester *et al.*, 2008).

The amount of serum calcium that is bound to plasma protein can be impacted by a number of factors, one of which is the pH of blood. Metabolic acidosis is associated with increased urinary calcium excretion and related sequelae, including nephrocalcinosis and nephrolithiasis. The increased urinary calcium excretion induced by metabolic acidosis predominantly results from increased mobilization of calcium out of bone and inhibition of calcium transport processes within the renal tubule. The net movement of calcium from bone into blood leads to excess calcium being excreted in urine, in an effort to stabilize systemic calcium concentrations. Metabolic acidosis increases ionized calcium in blood, by decreasing the amount bound to albumin. Furthermore, metabolic acidosis causes alterations in the renal reabsorptive capacity for calcium due to the direct inhibition of calcium transport within the nephron; this is evidenced by classic human studies where metabolic acidosis was induced and urinary calcium wasting observed, despite a fall in the filtered load of calcium (Alexander *et al.*, 2016; Kraut *et al.*, 2018; Kraut *et al.*, 2017). Phosphorus levels were significantly lower ($p = 0.0001$) in patients with acute kidney disease as compared with patients with CKD. And this agreed with (Ozmen *et al.*, 2007).

The action of many substances on the kidney, bone, and digestive system, such as parathyroid hormone, fibroblast growth factor 23, and vitamin D, keeps serum phosphorus levels generally stable. People over the age of 50 are at a higher risk of bone disease, and this group includes a significant number of those who also have chronic renal disease. Maintaining normal serum phosphorus once kidney function has deteriorated to end-stage illness (Stage 5) necessitates dietary restrictions, phosphate-binding medicines, and dialysis. Nonetheless, normal serum phosphorus, remain unclear in many patients with Stage 5 renal failure, and researchers are pursuing new targets that may block intestinal phosphorus

transport to improve phosphatemanagement. As renal disease progresses, protecting and monitoring bone health should help manage serum phosphorus (Suki et al., 2016). When kidney function is reduced to the point that the glomerular filtration rate (GFR) falls below 30mL/min/1.73 m², this is referred to as Stage 4 chronic kidney disease (CKD). Serum phosphorus levels begin to rise at this time and continue to climb as these individuals progress to end-stage renal disease. (O'Seaghdha et al., 2011).

Biomarkers

NGAL as a biomarker of kidney disease patients

The study revealed that patients with kidney failure show significance difference ($p < 0.0001$) increase in Serum levels of NGAL in kidney failure patients (5.93 ± 1.17) as compared with healthy control group (1.83 ± 0.59) that explained in figure (1). This was compatible with (Moriya et al 2017; Rusul, 2020) a study indicated that plasma NGAL was an important biomarker of interstitial lesions in CKD patients. Researchers also indicated that NGAL could have been used to estimate the end stage of risk levels for CKD. There was evidence that NGAL may also be used as a mediator of CKD progression (Viau et al; 2010). Previously, (Mishra et al; 2003) 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. More study has showed that there are several molecular forms of NGAL in urine, including a monomeric form produced by kidney tubular epithelial cells and a dimeric form produced by neutrophils. As a result of this variation, NGAL has the potential to increase the specificity of NGAL as a renal biomarker (Cai et al; 2010).

NGAL, is also defined as siderocalin, lipocalin-2 or lipocalin, is prevalent at multiple location, In the human Leukocytes were the main source of its expression originally isolated from neutrophils in addition to other tissues such as liver, epithelial cells, kidney particularly from nephrons loop of Henle and the collecting ducts (Wasung, et al. 2015; De Silva, et al; 2021). Diabetic nephropathy (DN) is a common diabetes complication caused by adverse renal tubular injury, which plays a critical part in the development and progression of DN, and renal tubular epithelial cell apoptosis contributes to kidney dysfunction, elevated incidence of serum creatinine, blood urea nitrogen, urine total protein/urine creatinine, and microalbuminuria, and decreased creatinine clear rate. (Dabla et al; 2010). Several studies have demonstrated the importance of urine NGAL as a predictive biomarker in diabetic nephropathy, but few have investigated the role of serum NGAL in these individuals. (Fu, 2012). In DMT2 patients, NGAL was not significantly raised in the early stages of diabetic nephropathy with Normal-Albuminuria and microalbuminuria. Another cross-sectional research of DMT1 individuals found no link between urine NGAL levels and GFR (Kim et al; 2012).

Cystatin C as a biomarker of kidney disease patients

The results in figure(4-4) revealed that patients with kidney failure show significance difference ($p < 0.05$) increase in Cystatin c levels in patients with Chronic (1.96 ± 0.78) and Acute kidney disease (1.98 ± 0.97) as compared with healthy control group (0.67 ± 0.38) and this is compatible with (Peralta, Shlipak, et

al., 2011) they indicated that Adding cystatin C to the combination of creatinine and ACR measures improved the predictive accuracy for all-cause mortality and end-stage renal disease. Researchers found that The presence of $eGFR < 60$ ml/min per 1.73 m² was related with an increased risk of mortality, cardiovascular events, and heart failure if verified by cystatin C. Individuals with reduced GFR due to creatinine alone had the same risks as those with normal GFR. Thus, cystatin C may play an essential clinical role in differentiating "greater risk" and "lowerrisk" patients for CKD problems.(Peralta, Katz, et al., 2011).

The study also indicated that there was no significance difference ($p > 0.05$) in cystatin c levels between AKI and CKD as seen in figure (5). The key benefits of a plasma cystatin C-based technique include free filtration and catabolizing in the proximal tubule, as well as independence from patient factors such as gender, age, or body mass (Filler et al., 2005). In several studies and meta-analyses, cystatin c has been shown to be more accurate than creatinine in estimating GFR, particularly in individuals with near-normal renal function. (Laterza et al., 2002; Dharnidhaka et al., 2002) It was demonstrated that estimating GFR using equations based on or serum cystatin C or serum creatinine alone was equally accurate, but combining these two might result in higher accuracy. In a meta-analysis of 17 studies involving 2521 individuals with CKD, found significant correlations between cystatin C, serum creatinine, and GFR. Finally, it was demonstrated that GFR estimate using cystatin C was more sensitive but less specific than GFR estimation using serum creatinine (Zhang et al., 2013).

Serum NGAL correlated with other studied parameters in Kidney failure Patients

Our study revealed high positive correlation between serum NGAL and creatinine ($r = 0.456, p\text{-value} < 0.001$) as showed in figure (2). The results also indicated there was positive correlation between NGAL and Urea as showed in figure (3) ($r = 0.498, P\text{-value} < 0.001$). the results also show high negative correlation between serum NGAL and $eGFR$ ($r = 0.321, p < 0.01$) as showed in figure(4). Malyszko and his college indicated that serum NGAL is strongly correlated with serum creatinine ($r = 0.78$) (Malyszko et al., 2009). The results in table (2) also show no significance correlation between serum NGAL and serum Calcium ($r = 0.098, p = 0.438$), phosphorus ($r = 0.113, p\text{-value} = 0.369$), B.Sugar ($r = -0.205, p\text{-value} < 0.292$).

Serum Cystatin C correlated with other studied parameters

Our study show negative correlation between Cystatin C and GFR ($r = -0.312, p < 0.01$) as showed in figure (6), the results also shows no significance correlation between Cystatin C and Urea ($R = 0.219, p = 0.08$) as showed in figure (7). The results also show positive correlation between Cystatin C and Creatinine in patients with kidney disease ($R = 0.326, p < 0.01$) as showed in figure (8), the results also indicated there was positive correlation between NGAL and Cystatin C in male patients with kidney disease ($R = 0.59, p < 0.01$) as showed in figure (9). The results in table (2) show no significance correlation between serum Cystatin c and serum calcium ($r = 0.037, p = 0.770$), serum phosphorus ($r = 0.107, p = 0.395$), B. SUGAR ($r = -0.001, p = 0.993$). Early detection of reduced renal function based on GFR evaluation is critical since it leads to renal failure; nevertheless,

overestimation or underestimating of real GFR may result in inadequate therapy or the scheduling of an inappropriate intervention (Lamb et al., 2005). In this regard, using simple and accurate GFR estimation approach in order to evaluate renal function accurately might be of great clinical significance. Recently, there has been a strong focus on the use of plasma cystatin C-based approaches. Because the plasma level of this agent can rise earlier than that of other materials such as serum creatinine, it may be more useful than the latter for the early diagnosis of renal failure. (Sjostrom et al., 2005; Massey, 2004).

Table 1
Biochemical and hematological parameters in CKD patients compared with the healthy control

| Groups | Parameters | Mean \pm SD | | p-value |
|-----------------------------------|------------|--------------------|----------------------|----------|
| | | KD Patients n=65 | Healthy control n=25 | |
| Age (year) | | 44.38 \pm 14.59 | 48.68 \pm 16.37 | 0.230 |
| Dialysis duration (m) | | 6 (0-30) # | 0 | NA |
| GFR (ml/min/1.73 m ²) | | 13.42 \pm 13.83 | 114.12 \pm 12.49 | 0.0001 * |
| Urea (mg/dl) | | 156.6 \pm 53.97 | 26.76 \pm 6.26 | 0.0001 * |
| Creatinine (mg/dl) | | 8.57 \pm 4.25 | 0.7 \pm 0.12 | 0.0001 * |
| Ca (mg/dl) | | 6.82 \pm 1.41 | 9.17 \pm 0.56 | 0.0001 * |
| PO (mg/dl) | | 7.99 \pm 4.03 | 3.49 \pm 0.52 | 0.0001 * |
| B. sugar (mg/dl) | | 177.92 \pm 74.11 | 93.99 \pm 10.75 | 0.0001 * |
| NGAL (ng/ml) | | 5.93 \pm 1.17 | 1.83 \pm 0.59 | 0.0001 * |
| Cystatin C (ng/ml) | | 1.97 \pm 0.78 | 0.67 \pm 0.38 | 0.0001 * |

*Significance difference at p-value<0.01 , Kidney disease patients= 65, healthy group=25,NA= not applicator

Table 2
Correlations between biomarkers and different biochemical parameters in male patients with kidney failure

| Biomarker | Parameters | NGAL (ng/ml) | Cystatin C (ng/ml) |
|------------|------------|--------------|--------------------|
| Ca (Mg/dl) | r | 0.098 | 0.037 |
| | p-value | 0.438 | 0.770 |
| | N | 65 | 65 |
| Po (Mg/dl) | r | 0.113 | 0.107 |
| | p-value | 0.369 | 0.395 |
| | N | 65 | 65 |
| B. SUGAR | R (Mg/dl) | -0.205 | -0.001 |
| | P-value | 0.292 | 0.993 |
| | N | 65 | 65 |

*significant differences at p-value <0.05

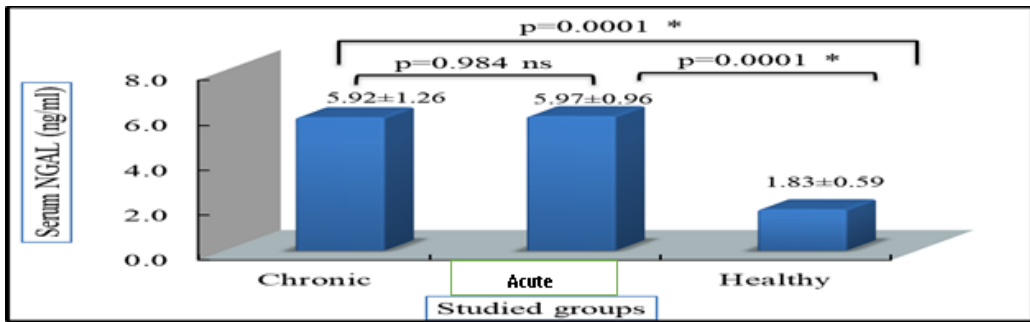


Figure 1. serum NGAL level in chronic and acute kidney disease patients compared with the healthy control groups. Chronic n=45, Acute n=20, and healthy control n=25

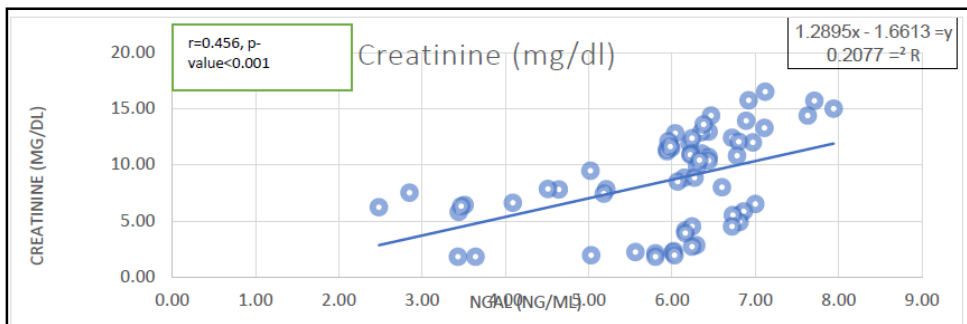


Figure 2. Significant positive correlation of serum creatinine with serum NGAL level in male patients with kidney failure

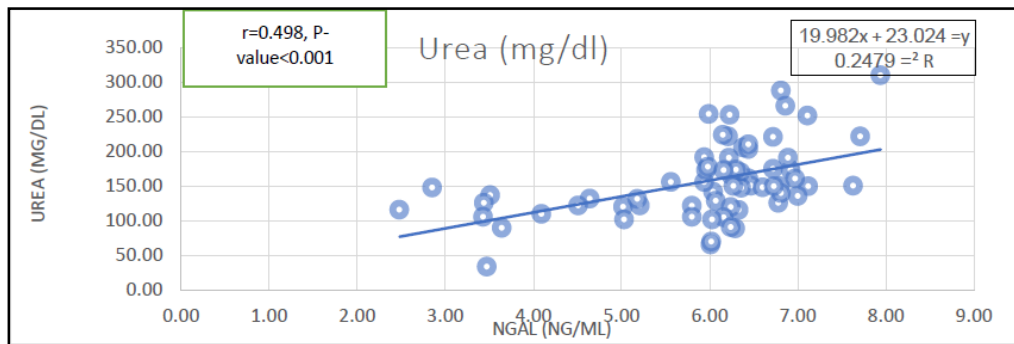


Figure 3. Significant positive correlation of serum urea with serum NGAL level in Kidney Disease patients

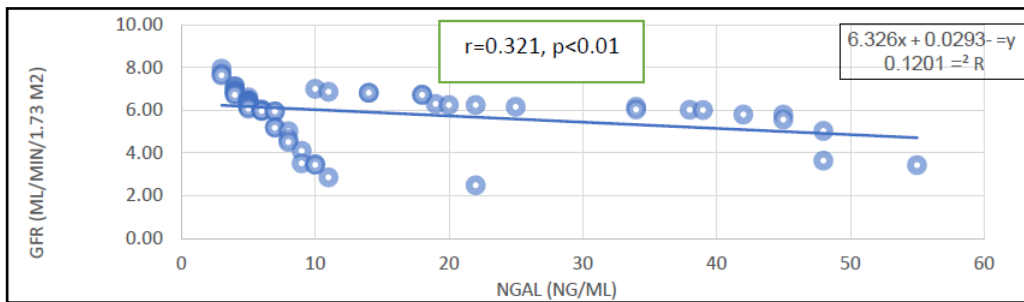


Figure 4. significant negative correlation of GFR with serum NGAL level in CKD patients

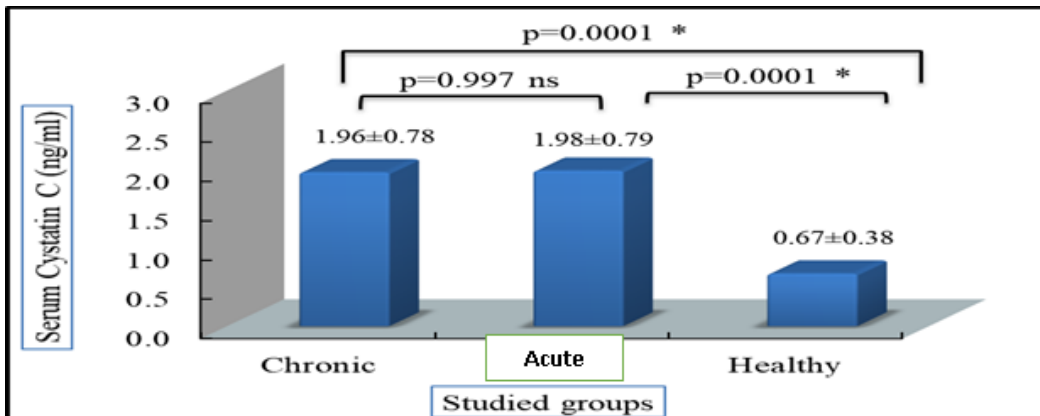


Figure 5. Serum Cystatin C level in chronic and acute of CKD patients compared with the healthy control groups. Chronic n=45, Acute n=20, and healthy control n=25

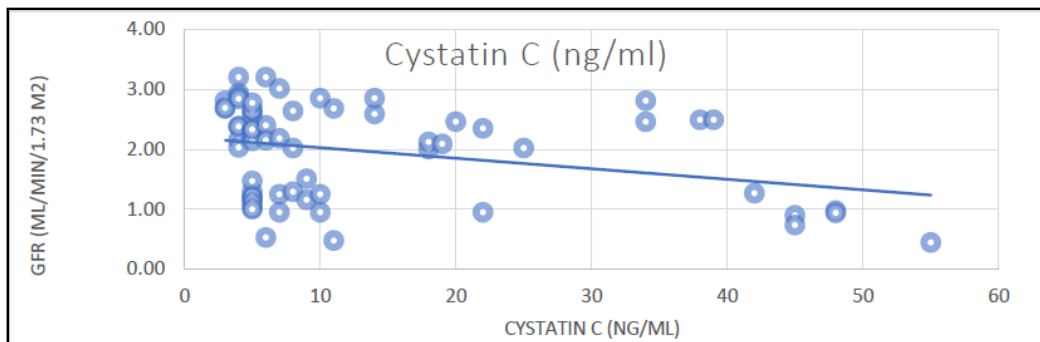


Figure 6. Significance negative correlation between GFR and Cystatin C in male patients with kidney disease. $r = -0.312, p < 0.01$

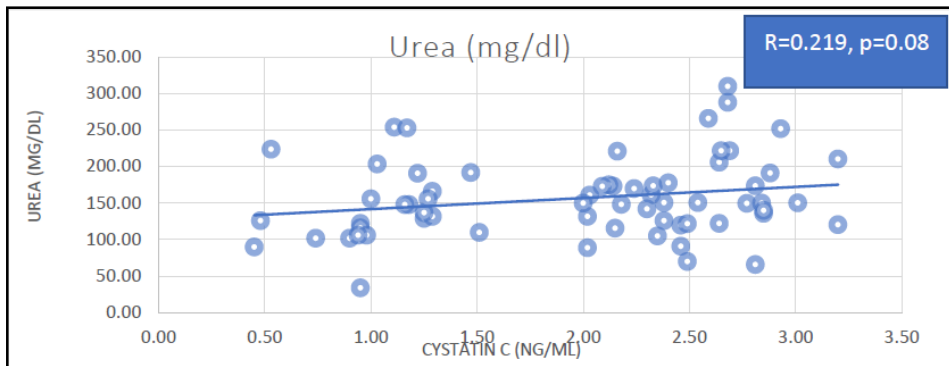


Figure 7. No significance correlation between Urea and Cystatin C in male patients with kidney disease. $R=0.219$, $p=0.08$

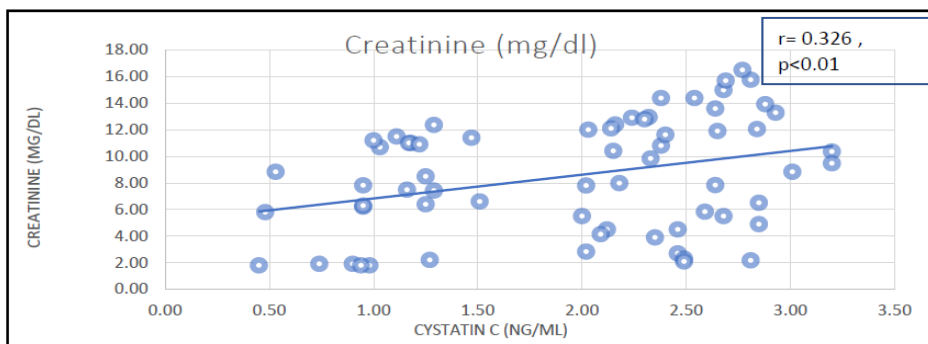


Figure 8. Positive correlation between Cystatin C and Creatinine in patients with kidney disease. $R=0.326$, $p<0.01$

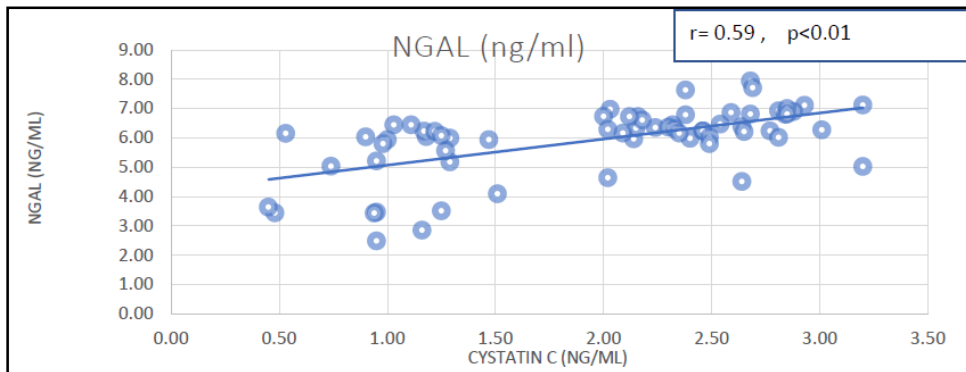


Figure 9. positive correlation between NGAL and Cystatin C in male patients with kidney disease. $R=0.59$, $p<0.01$

Conclusion

Serum NGAL and Cystatin C levels are significant biomarkers for predicting progressive kidney disease irrespective of recognized risk factors and these biomarkers are an effective tool for investigating patients at high risk of GFR reduction and may serve as an enrichment marker for clinical groups aimed at preventing CKD development.

References

- Abhisek, P. A., Panda, R., Mohapatra, J., Mohapatra, N., & Mohanty, S. (2016). Antihypertensive drug utilisation pattern among chronic kidney disease patients undergoing maintenance dialysis in a tertiary care teaching hospital. *Hypertension*, *100*,100.
- Alexander, R. T., Cordat, E., Chambrey, R., Dimke, H., & Eladari, D. (2016). Acidosis and Urinary Calcium Excretion: Insights from Genetic Disorders. *Journal of the American Society of Nephrology*,
- Bobulescu, I. A., & Moe, O. W. (2012). Renal transport of uric acid: evolving concepts and uncertainties. *Advances in chronic kidney disease*, *19*(6), 358–371. <https://doi.org/10.1053/j.ackd.2012.07.009>
- Buonafine, M., Martinez-Martinez, E., & Jaisser, F. ric. (2018). More than a simple biomarker: The role of NGAL in cardiovascular and renal diseases. *Clinical Science*, *132*(9), 909–923. <https://doi.org/10.1042/CS20171592>
- Cai, L., Rubin, J., Han, W., Venge, P., & Xu, S. (2010). The origin of multiple molecular forms in urine of HNL/NGAL. *Clinical Journal of the American Society of Nephrology*, *5*(12), 2229-2235.
- Dabla, P. K. (2010). Renal function in diabetic nephropathy. *World journal of diabetes*, *1*(2), 48.
- De Silva, P. M., Gunasekara, T. D. K. S. C., Gunarathna, S. D., Sandamini, P. M. M. A., Pinipa, R. A. I., Ekanayake, E. M. D. V., ... & Jayasundara, N. (2021). Urinary Biomarkers of Renal Injury KIM-1 and NGAL: Reference Intervals for Healthy Pediatric Population in Sri Lanka. *Children*, *8*(8), 684.
- De Silva, P. M., Gunasekara, T. D. K. S. C., Gunarathna, S. D., Sandamini, P. M. M. A., Pinipa, R. A. I., Ekanayake, E. M. D. V., ... & Jayasundara, N. (2021). Urinary Biomarkers of Renal Injury KIM-1 and NGAL: Reference Intervals for Healthy Pediatric Population in Sri Lanka. *Children*, *8*(8), 684.
- Dharnidharka, V. R., Kwon, C., & Stevens, G. (2002). Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *American journal of kidney diseases*, *40*(2), 221-226.
- Filler, G., Bökenkamp, A., Hofmann, W., Le Bricon, T., Martinez-Brú, C., & Grubb, A. (2005). Cystatin C as a marker of GFR—history, indications, and future research. *Clinical biochemistry*, *38*(1), 1-8.
- Gounden V, Bhatt H, Jialal I. Renal Function Tests. [Updated 2021 Jul 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK507821/>
- Gunasekara, T. D. K. S. C., De Silva, P. M., Herath, C., Siribaddana, S., Siribaddana, N., Jayasumana, C., ... & Jayasundara, N. (2020). The Utility of Novel Renal Biomarkers in Assessment of Chronic Kidney Disease of Unknown Etiology (CKDu): A Review. *International journal of environmental research and public health*, *17*(24), 9522.
- Huelin, P., Solà, E., Elia, C., Solé, C., Risso, A., Moreira, R., ... & Ginès, P. (2019). Neutrophil gelatinase-associated lipocalin for assessment of acute kidney injury in cirrhosis: a prospective study. *Hepatology*, *70*(1), 319-333.
- Kellum, J. A., Romagnani, P., Ashuntantang, G., Ronco, C., Zarbock, A., & Anders, H. J. (2021). Acute kidney injury. *Nature Reviews Disease Primers*, *7*(1). <https://doi.org/10.1038/s41572-021-00284-z>
- Kim, S. S., Song, S. H., Kim, I. J., Yang, J. Y., Lee, J. G., Kwak, I. S., & Kim, Y. K. (2012). Clinical implication of urinary tubular markers in the early stage of

- nephropathy with type2 diabetic patients. *Diabetes research and clinical practice*, 97(2), 251-257.
- Kraut, J. A., & Madias, N. E. (2017). Adverse effects of the metabolic acidosis of chronic kidney disease. *Advances in chronic kidney disease*, 24(5), 289-297.
- Kraut, J. A., & Nagami, G. T. (2018). Metabolic acidosis of chronic kidney disease. In *Textbook of Nephro-Endocrinology* (pp. 291-318). Academic Press.
- Lamb, E. J., Tomson, C. R., & Roderick, P. J. (2005). Estimating kidney function in adults using formulae. *Annals of clinical biochemistry*, 42(5), 321-345.
- Laterza, O. F., Price, C. P., & Scott, M. G. (2002). Cystatin C: an improved estimator of glomerular filtration rate?. *Clinical chemistry*, 48(5), 699-707.
- Levey, A. S., & Coresh, J. (2012). Chronic kidney disease. *The lancet*, 379(9811), 165-180.
- Liang, S., Shi, M., Bai, Y., Deng, Y., Fang, M., Li, J., Wu, Y., Peng, W., Hou, Y., Fang, H., Zhang, H., & Chen, C. (2020). The effect of glucocorticoids on serum cystatin C in identifying acute kidney injury: a propensity-matched cohort study. *BMC Nephrology*, 21(1), 1-10. <https://doi.org/10.1186/s12882-020-02165-1>
- Lim, L. M., Kuo, H. T., Kuo, M. C., Chiu, Y. W., Lee, J. J., Hwang, S. J., Tsai, J. C., Hung, C. C., & Chen, H. C. (2014). Low serum calcium is associated with poor renal outcomes in chronic kidney disease stages 3-4 patients. *BMC nephrology*, 15, 183. <https://doi.org/10.1186/1471-2369-15-183>
- Massey, D. (2004). Commentary: clinical diagnostic use of cystatin C. *Journal of clinical laboratory analysis*, 18(1), 55-60.
- Mishra, J., Ma, Q., Prada, A., Mitsnefes, M., Zahedi, K., Yang, J., ... & Devarajan, P. (2003). Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *Journal of the American Society of Nephrology*, 14(10), 2534-2543.
- Mishra, J., Ma, Q., Prada, A., Mitsnefes, M., Zahedi, K., Yang, J., ... & Devarajan, P. (2003). Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *Journal of the American Society of Nephrology*, 14(10), 2534-2543.
- Moriya, H., Mochida, Y., Ishioka, K., Oka, M., Maesato, K., Hidaka, S., ... & Kobayashi, S. (2017). Plasma neutrophil gelatinase-associated lipocalin (NGAL) is an indicator of interstitial damage and a predictor of kidney function worsening of chronic kidney disease in the early stage: a pilot study. *Clinical and experimental nephrology*, 21(6), 1053-1059.
- O'Seaghdha, C. M., Hwang, S. J., Muntner, P., Melamed, M. L., & Fox, C. S. (2011). Serum phosphorus predicts incident chronic kidney disease and end-stage renal disease. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, 26(9), 2885-2890. <https://doi.org/10.1093/ndt/gfq808>
- Ozmen, S., Danis, R., Akin, D., Cil, T., & Yazanel, O. (2007). Parathyroid hormone as a marker for the differential diagnosis of acute and chronic renal failure. *Renal Failure*, 29(4), 509-512. <https://doi.org/10.1080/08860220701275006>
- Ozmen, S., Danis, R., Akin, D., Cil, T., & Yazanel, O. (2007). Parathyroid hormone as a marker for the differential diagnosis of acute and chronic renal failure. *Renal Failure*, 29(4), 509-512. <https://doi.org/10.1080/08860220701275006>

- Peralta, C. A., Katz, R., Sarnak, M. J., Ix, J., Fried, L. F., De Boer, I., ... & Shlipak, M. G. (2011). Cystatin C identifies chronic kidney disease patients at higher risk for complications. *Journal of the American Society of Nephrology*, 22(1), 147-155.
- Peralta, C. A., Shlipak, M. G., Judd, S., Cushman, M., McClellan, W., Zakai, N. A., Safford, M. M., Zhang, X., Muntner, P., & Warnock, D. (2011). Detection of chronic kidney disease with creatinine, cystatin c, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA - Journal of the American Medical Association*, 305(15), 1545-1552. <https://doi.org/10.1001/jama.2011.468>
- Rusul, J. A. (2020). Evaluation of some biomarkers as a predictable of risk factors in patients with chronic kidney disease in Al-Najaf province, Iraq .
- Rysz, J., Gluba-Brzózka, A., Franczyk, B., Jablonowski, Z., & Cialkowska-Rysz, A. (2017). Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. *International Journal of Molecular Sciences*, 18(8). <https://doi.org/10.3390/ijms18081702>
- Schaalan, M. F., & Mohamed, W. A. (2016). Determinants of hepcidin levels in sepsis-associated acute kidney injury: Impact on pAKT/PTEN pathways?. *Journal of immunotoxicology*, 13(5), 751-757.
- Sjostrom, P., Tidman, M., & Jones, I. (2005). Determination of the production rate and non-renal clearance of cystatin C and estimation of the glomerular filtration rate from the serum concentration of cystatin C in humans. *Scandinavian journal of clinical and laboratory investigation*, 65(2), 111-124.
- Suki, W. N., & Moore, L. W. (2016). Phosphorus Regulation in Chronic Kidney Disease. *Methodist DeBakey cardiovascular journal*, 12(4 Suppl), 6-9. <https://doi.org/10.14797/mdcj-12-4s1-6>
- Tuttle, K. R., Alicic, R. Z., Duru, O. K., Jones, C. R., Daratha, K. B., Nicholas, S. B., McPherson, S. M., Neumiller, J. J., Bell, D. S., Mangione, C. M., & Norris, K. C. (2019). Clinical Characteristics of and Risk Factors for Chronic Kidney Disease Among Adults and Children: An Analysis of the CURE-CKD Registry. *JAMA Network Open*, 2(12), e1918169. <https://doi.org/10.1001/jamanetworkopen.2019.18169>
- Viau, A., El Karoui, K., Laouari, D., Burtin, M., Nguyen, C., Mori, K., ... & Terzi, F. (2010). Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. *The Journal of clinical investigation*, 120(11), 4065-4076.
- Viau, A., El Karoui, K., Laouari, D., Burtin, M., Nguyen, C., Mori, K., ... & Terzi, F. (2010). Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. *The Journal of clinical investigation*, 120(11), 4065-4076.
- Wasung, M. E., Chawla, L. S., & Madero, M. (2015). Biomarkers of renal function, which and when?. *Clinica chimica acta*, 438, 350-357.
- Worcester, E. M., & Coe, F. L. (2008, March). New insights into the pathogenesis of idiopathic hypercalciuria. In *Seminars in nephrology* (Vol. 28, No. 2, pp. 120-132). WB Saunders.
- Zhang, M., Cao, X., Cai, G., Wu, D., Wei, R., Yuan, X., ... & Chen, X. (2013). Clinical evaluation of serum cystatin C and creatinine in patients with chronic kidney disease: a meta-analysis. *Journal of international medical research*, 41(4), 944-955.