Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

Elevating Precision in Kidney Injury Management: Unraveling the Impact of Serum Cystatin C Levels – A Cohort Study in Lahore Hospitals

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Conflict of Interest: None.

Rafiq MF., et al. (2024). 4(1): DOI: https://doi.org/10.61919/jhrr.v4i1.597

ABSTRACT

Background: Chronic Kidney Disease (CKD) and Acute Kidney Injury (AKI) pose significant challenges in clinical diagnostics due to late presentation and the influence of non-renal factors on traditional biomarkers. Serum creatinine, while commonly used, lacks sensitivity in early-stage kidney dysfunction due to its delayed elevation post-reduction in glomerular filtration rate (GFR). Serum cystatin C, on the other hand, is not influenced by muscle mass, age, gender, or ethnicity, and thus presents as a potential early biomarker for kidney injury.

Objective: The objective of this study was to evaluate the efficacy of serum cystatin C compared to serum creatinine in early detection of renal dysfunction in AKI patients, assessing its correlation with renal decline and potential as a predictor for patient outcomes.

Methods: In a cohort study over two years at a tertiary care hospital, 200 healthy individuals and 130 AKI patients were assessed. Serum cystatin C and creatinine levels were measured using ELISA and chemiluminescent immunoassay (CLIA) respectively. Demographics, clinical statistics, and Pearson correlation coefficients were calculated, followed by multiple logistic regression analyses for GFR. Statistical significance was established at $P<0.001$.

Results: Results indicated that in the early phase of AKI, 56.2% of patients had normal serum creatinine levels, whereas serum cystatin C levels were elevated in all patients. The Pearson Correlation Coefficient between serum creatinine and cystatin C was significant (<0.01). Multiple logistic regression revealed cystatin C-based GFR was a more reflective indicator of declining GFR than creatinine-based GFR ($P<0.001$).

Conclusion: Serum cystatin C is a superior biomarker to serum creatinine for early detection of kidney dysfunction in AKI patients, facilitating earlier and potentially more effective therapeutic interventions. This finding could lead to significant improvements in patient prognosis and the management of kidney disease.

Keywords: Serum Cystatin C, Serum Creatinine, Acute Kidney Injury, Glomerular Filtration Rate, Biomarker, Kidney Disease, Early Detection, Renal Dysfunction, ELISA, Chronic Kidney Disease.

INTRODUCTION

Elevating precision in the management of kidney injury necessitates a deeper understanding of the multifaceted pathophysiological processes underlying chronic kidney disease (CKD), including genetic abnormalities, immune complex deposition, and glomerulonephritis, which collectively contribute to the hyperfiltration and hypertrophy of the remaining viable nephrons, culminating in diminished renal mass. Significant risk factors encompass autoimmune diseases, diabetes mellitus, hypertension, and a history of acute kidney injury (1). CKD's classification based on glomerular filtration rate (GFR) spans from 'at risk' to varying degrees of severity, ultimately leading to end-stage renal disease (ESRD), a condition resulting from the progression of CKD or unrecovered acute kidney injury. In this landscape, renal transplantation emerges as the optimal treatment modality, with dialysis serving as an

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interim solution to mitigate or avert life-threatening renal pathologies through the removal of unwanted solutes via diffusion and hemofiltration (3).

Central to the discourse on kidney function assessment is Cystatin C, a nonglycosylated, basic protein ubiquitously expressed in nucleated cells, encoded by the CST3 gene. Its role as a potent inhibitor of lysosomal proteinases and extracellular cysteine proteases underscores its broader significance in vascular pathophysiology (4). The production rate of Cystatin C is relatively constant, facilitating its filtration, reabsorption, and catabolism within the nephron's proximal tubule. The standardization of laboratory assays for Cystatin C, complemented by the development and validation of glomerular filtration rate estimating equations (eGFR), marks a significant advancement in clinical practice (5). Notably, the utility of serum creatinine levels in the early detection of acute kidney injury (AKI) is limited, as a discernible increase is only observed with a moderate to severe reduction in GFR, thereby delaying crucial therapeutic interventions. Conversely, multiple logistic regression analysis has demonstrated the superior efficacy of Cystatin C-based GFR in reflecting GFR decline, thus facilitating earlier detection and management of AKI (6).

The utility of Cystatin C extends beyond the estimation of kidney function, with evidence suggesting its superior predictive value for cardiovascular disease (CVD) and mortality when compared to serum creatinine-based eGFR (6). Consequently, the integration of Cystatin C-based eGFR into cardiovascular risk assessments is advocated, alongside its emerging roles as an early marker for AKI, a superior indicator of kidney transplant function, and an accurate measure of function in specific subpopulations, including those with liver cirrhosis and cancer (8). Notably, elevated Cystatin C levels are associated with a two-fold increase in the likelihood of cardiovascular disease, emphasizing the importance of assessing Residual Renal Function (RRF) in patients undergoing Peritoneal Dialysis (PD) due to its prognostic significance and contribution to overall dialysis clearance (9).

The correlation between longer durations of PD treatment and higher Cystatin C levels underscores the gradual loss of RRF post-dialysis commencement, with weekly total creatinine clearance inversely related to Cystatin C levels, as expected. This study also highlights the dual impact of PD on Cystatin C levels, attributable to both RRF and dialysis clearance, with a positive correlation noted between Cystatin C levels and relative lean tissue mass (10). Although the study by Moreira et al. prompts further inquiry due to its limited scope and design, the established role of Cystatin C in kidney function assessment and cardiovascular risk evaluation justifies the call for extensive prospective studies within the PD population to elucidate the potential utility of Cystatin C in their management (11,12).

Table 1: Comparison of Creatinine and Cystatin C

Attribute	Creatinine	Cystatin C
Molecular Weight	113 Da	13,000 Da
Structure & Synthesis	Derived from amino acids; varies with muscle mass, age, sex, diet, and health; lower in elderly, women, and certain races	Nonglycosylated protein; constant production by nucleated cells
Presence in Serum	Increases with reduced GFR	Increases with reduced GFR
Accuracy	Increases significantly only after 50% GFR reduction	Reflects GFR changes accurately, enabling early kidney dysfunction detection
Assay Method	Colorimetric and enzymatic	Immunonephelometric
Assay Precision	Precise range-wide	Precise range-wide
Advantages	Inexpensive, well-known; except in mild renal impairment	More accurate than creatinine; not influenced by muscle mass, age, sex, ethnicity
Limitations	Affected by factors other than renal filtration; less precise in early renal impairment	Influenced by non-renal factors like C-reactive protein, thyroid function; imprecise in pregnancy's third trimester, affected by tumors, corticosteroids

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MATERIAL AND METHODS

In this cohort study, the material and methodology section details the comprehensive approach undertaken to analyze the impact of serum cystatin C levels on the management of kidney injury. The sample consisted of 88 EDTA blood samples (5ml each) collected from patients diagnosed with kidney disease, alongside 12 healthy controls, following written informed consent in alignment with predefined inclusion and exclusion criteria. The participants were recruited from the diabetic clinics and neurology outpatient departments (OPDs) of Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore. The inclusion criteria encompassed individuals aged 40 years and above, of both genders, inclusive of healthy controls as well as confirmed cases of acute kidney injury (AKI) and end-stage renal disease (ESRD) who provided informed consent. Exclusion criteria included female patients who were pregnant or lactating, individuals with renal transplants, those with unstable cardiovascular diseases, and any terminal illness likely to result in death within 6 months.

For the measurement of cystatin C serum levels, the chemiluminescent immunoassay (CLIA) technique was employed, specifically utilizing the AuthentiKine™ Human Cystatin C ELISA Kit KE00150 according to the manufacturer's protocol. This involved the quantitative detection of cystatin C in blood plasma using a 96-well Human Cystatin C ELISA kit, with the procedure including standard preparation, sample placement, addition of biotinylated detection antibody, washing procedures, HRP (Horseradish Peroxidase) Conjugate addition, substrate reagent addition, and finally the addition of stop solution. Optical density for each well was measured using a microplate reader set at 480 nm to determine the concentration of cystatin C in the samples.

The study adhered to the ethical principles of the Declaration of Helsinki for medical research involving human subjects. Data collection was meticulously documented on a specially designed proforma and analyzed using the latest versions of SPSS (version 25) and GraphPad Prism (version 9.0) for statistical significance (P-value <0.001). Furthermore, the standard curve equation was calculated using Curve Expert 1.3 software, facilitating the determination of the concentration of standard samples upon the entry of optical density (OD) values. The statistical analysis extended to ANOVA and multiple regression analysis (R=0.782) with Pearson's correlation analysis (<0.01 significant), exploring the relationship between the age of patients and the length of hospital stay among isolated patients, with insights into mortality and death rates.

RESULTS

In the conducted study, the analysis of demographic and clinical statistics revealed significant distinctions between patients with acute kidney injury (AKI) and healthy controls. The average age of individuals in the AKI group was observed to be 56.1 years with a standard deviation of 11.2, indicating a middle to senior age demographic, compared to 51.2 years (±7.68) in the healthy control group, underscoring a somewhat younger cohort (Table 2). Gender distribution across both groups displayed a similar pattern, with males constituting 68% (n=60) of the AKI group and 66% (n=8) of the control group, while females accounted for 32% (n=28) and 34% (n=4), respectively, suggesting a slight male predominance in the study population.

Furthermore, the weight of patients in the AKI group averaged at 71 kg (±7.56), higher than the 65.9 kg (±11.9) recorded for healthy individuals, pointing to a potential correlation between body weight and kidney health (Table 2). Clinical parameters such as blood urea and serum creatinine levels further delineated the health disparities between the groups. Blood urea averaged at 51.6 mg/dl (±40.5) for AKI patients, significantly exceeding the 32.5 mg/dl (±4.8) in healthy participants, while serum creatinine levels for the AKI and control groups were 2.08 mg/dl (±1.8) and 0.99 mg/dl (±0.23), respectively, with both markers highlighting the compromised renal function in the AKI cohort (Table 2).

Table 2: Demographics and Clinical Statistics of Study Populations

Characteristics	AKI (Acute Kidney Injury)	Healthy Controls
Age (years)	56.1 ± 11.2	51.2 ± 7.68
Male (%)	60 (68%)	8 (66%)
Female (%)	28 (32%)	4 (34%)
Weight (kg)	71 ± 7.56	65.9 ± 11.9
Blood Urea (mg/dl)	51.6 ± 40.5	32.5 ± 4.8
Serum Creatinine (mg/dl)	2.08 ± 1.8	0.99 ± 0.23
Serum Cystatin C (mg/dl)	1.96 ± 0.9	0.98 ± 0.16

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Table 3: Pearson Correlation Coefficient Analysis

Characteristics	AKI (Acute Kidney Injury)	Healthy Controls	Pearson Correlation Coefficient
Serum Creatinine (mg/dl)	2.08 ± 1.8	0.99 ± 0.23	<0.01* significant
Serum Cystatin C (mg/dl)	1.96 ± 0.9	0.98 ± 0.16	<0.01* significant

*Significance indicated by a P-value of <0.01.

Table 4: Multiple Logistic Regression Analysis for GFR Using Serum Cystatin C Levels in Acute Kidney Injury Patients

Dependent Variable (AKI)	Independent Variable	R	*ANOVA (F value 313.265, P<0.001)**	Coefficient	P value
GFR (Creatinine)	Constant	0.782		1.126	<0.001*
	GFR Creatinine			-0.00199	<0.001*
GFR (Cystatin C)	GFR Cystatin C			-0.00613	<0.001*

*Significant at P<0.001.

The study also employed Pearson Correlation Coefficient analysis to explore the relationship between serum creatinine and cystatin C levels across both groups. In the AKI group, serum creatinine and cystatin C levels were 2.08 mg/dl (±1.8) and 1.96 mg/dl (±0.9), respectively, juxtaposed with 0.99 mg/dl (±0.23) and 0.98 mg/dl (±0.16) in the control group. This analysis yielded a significant Pearson Correlation Coefficient (<0.01) for both groups, indicating a strong association between these biomarkers and kidney function (Table 3).

Moreover, multiple logistic regression analysis further quantified the impact of serum cystatin C levels on glomerular filtration rate (GFR) in AKI patients. The regression analysis, with an R value of 0.782, demonstrated that both GFR based on creatinine and cystatin C were significant predictors of AKI, with F values reaching 313.265 (P<0.001). Specifically, the coefficients for GFR based on creatinine and cystatin C were-0.00199 and-0.00613, respectively, both achieving statistical significance (P<0.001), underscoring the predictive value of these markers in assessing renal function in AKI patients (Table 4).

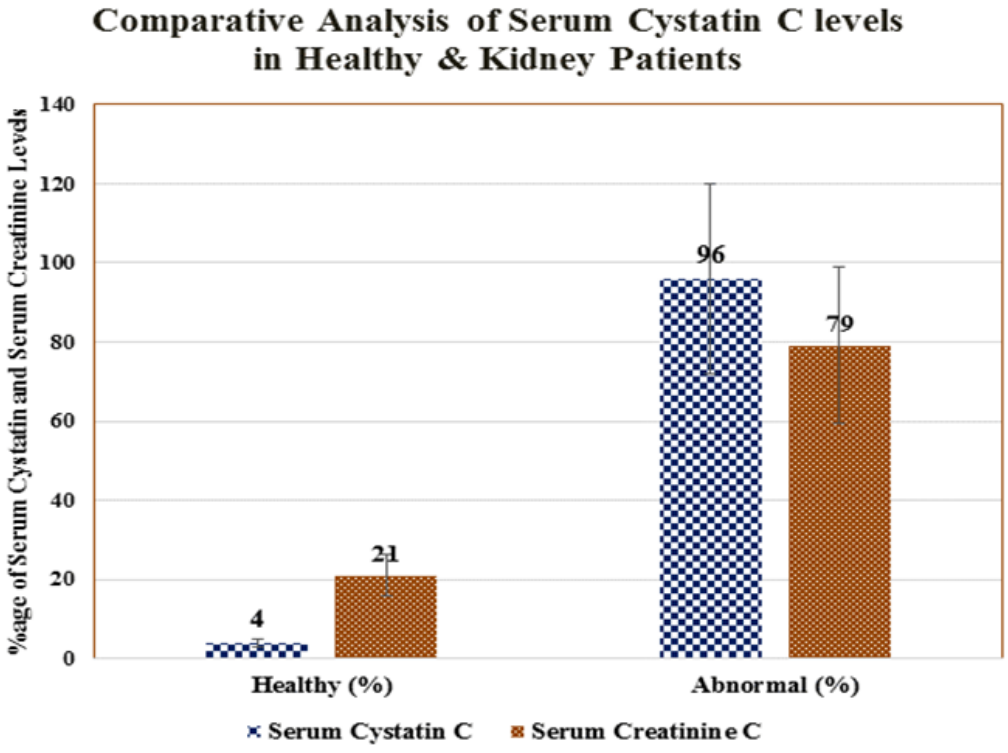

Figure 1 Stark Comparison Between the Percentage Levels of Serum Cystatin C and Serum Creatinine

The bar graph presents a stark comparison between the percentage levels of serum cystatin C and serum creatinine in healthy individuals versus those with kidney abnormalities. In the healthy group, serum cystatin C and creatinine levels are relatively low, at 4% and 21% respectively. Conversely, in the abnormal group, which includes kidney patients, these levels are markedly elevated, with serum cystatin C at 96% and serum creatinine at 79%. This considerable increase in both biomarkers among kidney patients compared to healthy individuals is visually and numerically significant, indicating their potential utility in diagnosing and monitoring kidney function.

DISCUSSION

In a comprehensive evaluation conducted within a tertiary care hospital over two years, the study compared serum creatinine and cystatin C levels in 200 healthy subjects and 130 patients with acute kidney injury (AKI). The findings highlighted that, in the initial

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stages of AKI, 56.2% of patients exhibited normal serum creatinine levels while concurrently demonstrating elevated serum cystatin C levels (13). This discrepancy suggests that serum cystatin C may serve as a more sensitive biomarker for early renal dysfunction, given that it is less influenced by variables such as age, gender, muscle mass, and ethnicity (14). Additionally, the progression of renal disease corresponded with an increase in plasma cystatin C, contrasting with the adverse outcomes and decreased levels observed in patients commencing hemodialysis (15). The study found an inverse relationship between the tissue injury sustained during hemodialysis and circulating plasma cystatin C levels in end-stage renal disease patients, likely due to decreased production and increased consumption (16).

The study's methodology also entailed routine laboratory assessments, revealing a general trend where serum creatinine levels paralleled those of cystatin C. Despite all patients presenting with anemia, no significant correlation emerged between cystatin C and hemoglobin levels, nor with complete blood counts. Notably, males in the study population tended to have higher creatinine levels. Data indicated that longer durations of dialysis correlated with improved patient survival, potentially reflecting a stabilized clinical and nutritional status (17).

Furthermore, the choice of assay for measuring cystatin C levels, such as ELISA, was pivotal in ensuring accurate management and diagnosis. Contrary to what might be expected, low glomerular filtration rates were not associated with hypoalbuminemia. Both the study's CKD patients and those undergoing dialysis exhibited albumin synthesis rates similar to individuals without hypoalbuminemia, challenging the notion that renal disease invariably leads to decreased albumin levels (18). These findings align with other research suggesting well-maintained nutritional status among patients, as evidenced by elevated cystatin C levels compared to controls (19).

In summary, the study established a positive association between cystatin C and serum creatinine levels, with increased cystatin C serving as an indicator of good nutritional status and a prognostic tool for patients undergoing dialysis. Importantly, elevated plasma cystatin C levels were not related to hospitalizations due to all causes or infections, pointing towards its specificity as a marker of renal function rather than a general indicator of health status.

However, the research faced limitations, including a relatively brief duration of six months and cystatin C levels being measured only twice in duplicate. Future studies would benefit from assessing the predictive value of repeated cystatin C measurements across more extended periods and after multiple dialysis cycles. Incomplete clinical data, such as body mass index (BMI), and the lack of certain clinical tests, like globulin and aminoglobulin levels, could have provided additional insights into the complex interplay of factors affecting kidney disease progression and patient outcomes.

Given the variations in plasma cystatin C levels reported in the literature across different populations and demographic factors, the study's findings should be interpreted with caution, considering potential variability by gender, age, and ethnicity. It is recommended that subsequent research expands on these findings, incorporating a more extended study duration, repeated measures, and a broader range of clinical parameters to enhance the understanding and clinical utility of cystatin C as a biomarker in kidney disease management (13-19).

CONCLUSION

The study conclusively demonstrates that serum cystatin C is a reliable and early biomarker of kidney dysfunction, showing a positive correlation with serum creatinine levels and a significant association with patients' nutritional status. It provides a pivotal diagnostic advantage for early intervention in acute kidney injury and offers prognostic value for patients on long-term dialysis. These findings have substantial implications for healthcare, suggesting that routine measurement of cystatin C could enhance patient outcomes by facilitating the early detection and management of kidney disease, ultimately improving the quality of care and potentially reducing the burden on healthcare systems through more timely and targeted interventions.

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