DOI: 10.7860/JCDR/2024/65414.19097

Original Article

Biochemistry Section

Cystatin C as an Early Marker of Renal Dysfunction in Patients with Cirrhosis of Liver: A Cross-sectional Study

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ABSTRACT

Introduction: Serum cystatin C has been proposed as a sensitive marker for detecting renal impairment. Renal impairment can often co-exist with liver cirrhosis, as decreased blood flow through the cirrhotic liver can lead to kidney dysfunction. However, its role in patients with liver cirrhosis has not been extensively studied, especially in the detection of early renal impairment.

Aim: To investigate whether serum cystatin C could serve as a potential marker for detecting early renal dysfunction in patients with liver cirrhosis.

Materials and Methods: This hospital-based cross-sectional study was conducted on 60 patients with liver cirrhosis at Assam Medical College and Hospital, Dibrugarh, Assam, India, from June 2019 to May 2020. All patients were assessed for clinical symptoms and laboratory parameters, focusing on renal function tests, measurement of serum cystatin C levels, and Glomerular Filtration Rate (GFR). A two-tailed p-value <0.05 was considered statistically significant in all calculations.

Results: In total, 60 consecutive patients with liver cirrhosis were evaluated during the study period, 85% of whom were males. The mean age was 47.4±9.3 years. The mean values of GFR calculated from the mean values of serum creatinine and serum cystatin C were 59.57±31.48 and 31.25±18.32, respectively, both showing a negative correlation (r=-0.802 and r=-0.817, respectively), indicating that cystatin C has a superior correlation with GFR when compared to serum creatinine. Regression analysis showed a tighter distribution of results around the regression line for cystatin C, as indicated by an R2 value of 0.67, compared to 0.64 for creatinine. It was also observed that for every unit rise in serum creatinine and cystatin C value, GFR decreased by 29.89 and 14.67, respectively.

Conclusion: Serum cystatin C is a better predictor of GFR than serum creatinine and can therefore be used as an early marker of renal dysfunction in the course of liver cirrhosis.

Keywords: Creatinine, Glomerular filtration rate, Hepato-renal syndrome, Renal function test

INTRODUCTION

Cirrhosis is a widespread distortion of the liver's internal structure that occurs when a large amount of normal liver tissue is permanently replaced with fibrotic tissue and regenerative nodules. It has a variety of clinical manifestations and complications, which often result in hospitalisation, impaired quality of life, and high mortality [1]. Cirrhosis of the liver is often accompanied by functional renal failure, which occurs due to alteration of various haemodynamic mechanisms, leading to sodium and water retention, and reduction in GFR [1].

The Hepato-renal Syndrome (HRS) is a functional form of renal failure that occurs in about 10% of cases of advanced liver cirrhosis [2]. HRS is classified into two types: Type 1 HRS is characterised by rapidly progressive impairment in renal function with at least a doubling of serum creatinine in less than 2 weeks, and in Type 2 HRS, there is a steady or slow deterioration of renal function [2].

HRS is defined by an increase in serum creatinine levels. However, the reliability of serum creatinine as a marker of renal dysfunction is limited due to multiple factors: i) decreased production of creatinine due to a decrease in muscle mass, poor nutrition, and impaired synthesis of creatine (the precursor of creatinine) from the liver; ii) increased elimination of creatinine by renal tubular secretion; iii) dilution of serum creatinine in the body due to the oedematous state; and iv) low interpretation of serum creatinine due to high serum bilirubin [3-5].

As a result, rather than relying on serum parameters, measurements of exogenous or endogenous substance clearance rates have been introduced. Inulin clearance is considered the gold standard among these but is rarely used due to costs and inconvenience. For hospitalised patients, the most commonly used parameter is creatinine clearance. However, it necessitates 24-hour urine collection and may be inaccurate in outpatient settings. Hence, a simple, more accurate serum parameter that is sensitive to minor changes in renal function and un-affected by external factors is required. Many such parameters have been introduced in the last decade, with cystatin C being the most promising [6].

Cystatin C is a non-glycosylated basic protein with a molecular weight of 13 kDa that is produced at a constant rate by all nucleated cells. It functions as an inhibitor of cysteine proteinase, is completely filtered by the glomerulus in the kidney, and is almost completely re-absorbed and catabolised by the proximal tubular cells [7]. Serum cystatin C has been shown to be more sensitive than creatinine for early detection of renal dysfunction [8], and it reflects GFR independent of muscle mass, serum bilirubin, and body composition- factors that are especially affected in patients with end-stage liver disease. Unlike serum creatinine, cystatin C is less affected by age, gender, or muscle mass, making it a more accurate biomarker for kidney function [9].

While cystatin C has been recognised as a useful marker for kidney function in various patient populations [7-9], its application and relevance in the context of liver cirrhosis seem to have been explored less extensively. In this current study, the primary objective is to assess the utility of serum cystatin C as an indicator of renal function in individuals diagnosed with liver cirrhosis. Furthermore, the authors here aim to investigate its potential as an early indicator of renal impairment in these patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted from June 2019 to May 2020 at the Department of General Medicine in Assam

Medical College and Hospital, Dibrugarh, Assam, India. The study was approved by the Institutes Ethics Committee (Reference No AMC/IEC/PG/1926 dated 07/10/2020), and informed written consent was obtained from all the participants.

Inclusion criteria: Patients aged 18-80 years (both males and females) diagnosed with cirrhosis of the liver based on clinical indicators (e.g., jaundice, ascites, muscle wasting, cutaneous spider angioma, ecchymosis, and palmar erythema), laboratory findings (e.g., decreased serum albumin and prolonged prothrombin time), and ultrasonographic findings (coarse echo pattern and nodular surface) were included [10].

Exclusion criteria: Patients with end-stage renal disease, hepatocellular carcinoma, congestive heart failure, sepsis, dehydration, a history of gastrointestinal bleeding during the month before enrollment, spontaneous bacterial peritonitis, hyperthyroidism, or hypothyroidism were excluded.

Sample size: According to the study by Choi YJ et al., the prevalence of renal dysfunction in cirrhosis is 16.8% [11]. Now, taking p as 16.8% and q as 83.2%, with an absolute error (d) of 10%, the sample size is calculated as 55.9 (approximately 60) using the formula for sample size (N)=Z^2PQ/d^2. After rounding off, the final calculated sample size was 60.

Procedure

Data regarding age, gender, history of hypertension, renal and cardiac diseases was collected from all the participants. Estimation of serum creatinine was performed by random sampling using the Vitros 5600 integrated system auto analyser by the enzymatic method. The reference range for serum creatinine levels was defined as 0.66 to 1.25 mg/dL for males and 0.52-1.04 mg/dL for females in the present study, serving as the established cut-off values for normalcy within this particular biomarker [12]. Serum cystatin C estimation was carried out using the semi-auto analyser MICROLAB 300 (Make-ELI Tech Group, Spankeren, Netherlands) with reagents supplied by Coral Clinical Systems, Tulip Diagnostics, India. The test principle is based on the immune-turbidimetric method. In this method, the cystatin C in the samples was mixed with cystatin C latex reagent. Insoluble aggregates are produced following the addition of the activation buffer.

The resultant turbidity thus produced is measured at 630 nm and quantified by serial dilution of the calibrator supplied by Tulip Diagnostics. The specified reference range for serum cystatin C in our study was delineated as 0.61 to 1.01 mg/L, establishing the range within which normal values for this biomarker were considered [13]. Samples were categorised based on the GFR (mL/min/1.73 m^2) (estimated by MDRD/CKD epi 2021 equation) according to the Kidney Disease Improving Global Outcome (KDIGO) classification of Chronic Kidney Disease (CKD), and values of cystatin C were compared with serum creatinine [14].

The equation from the Modification of Diet in Renal Disease (MDRD) study [15] used in the present study was as follows:

Estimated GFR (mL/min per 1.73 m^2): $1.86\times(S_{cr})^{-1.154}\times(age)^{-0.203}$

GFR was also determined using a straightforward calculation based on serum cystatin C levels, employing the formula: GFR=100/serum cystatin C (mg/L). This method offers a simplified yet effective approach for estimating GFR in the context of the present study [16].

STATISTICAL ANALYSIS

Continuous data were presented as mean (with standard deviation). Subsequently, unpaired t-tests were performed to compare the data among groups. Correlations between variables were assessed using the correlation coefficient according to Pearson (r) in Microsoft Office Excel 2007. Probability values (p) less than 0.05 were considered significant.

RESULTS

In this study, out of 60 patients, the mean age of the cirrhotic patients was found to be 47.4±9.3 years, with ages ranging from 21 years to 60 years. The maximum number of cases belonged to the age group 41-50 years. A total of 51 (85%) were male, while 9 (15%) were female, resulting in a male to female ratio of 5.6:1. Among the study population, the majority of patients (26, 43.3%) were in stage 2 of CKD, followed by stage 3 (21, 35%). Co-morbidities were reported in one-third of the participants, with diabetes being the most common (13, 21.7%), followed by hypertension (5, 8.3%). A significant portion of the participants had a history of alcohol intake (13, 21.7%) and smoking (23, 38.3%), indicating these as common risk factors [Table/Fig-1].

Parameters	Sub-divisions	Total N=60 n (%)
Age (years)	47.4±9.3 years	
	18-20	0.00
	21-30	5 (8.33%)
	31-40	20 (33.33%)
	41-50	21 (35.00%)
	51-60	14 (23.33%)
Gender	Male	51 (85%)
	Female	9 (15%)
CKD grade	**GFR categorises (mL/min/1.73 m^2) description and range**	
	G1 (90)	5 (8.3%)
	G2 (60-89)	21 (35%)
	G3a (45-59)	16 (26.7%)
	G3b (30-44)	10 (16.7%)
	G4 (15-29)	8 (13.3%)
	G5 (<15)	0
Co-morbidity	Diabetes mellitus	13 (21.7%)
	Hypertension	5 (8.3%)
	Autoimmune disease	2 (3.3%)
H/O smoking	23 (38.3%)	
Alcohol	13 (21.7%)	

[Table/Fig-1]: Demographic profile and baseline data of the study population.

In the current investigation, a robust negative correlation was established between cystatin C and GFR, with a statistically significant correlation coefficient of -0.817 (p<0.001). Notably, the strength of this correlation surpassed that of creatinine, which exhibited a slightly lower correlation coefficient of -0.802 (p<0.0001) with GFR. This finding underscores the potential superiority of cystatin C as a biomarker in assessing renal function compared to creatinine in the studied population [Table/Fig-2].

Biomarkers	Mean±SD	Pearson's correlation coefficient	p-value
Creatinine	1.62±0.85	r=-0.802	**<0.0001**
GFR based on creatinine	59.57±3 1.48		
Cystatin C	2.41±1.02	r=-0.817	**<0.0001**
GFR based on Cystatin C	31.25±18.32		

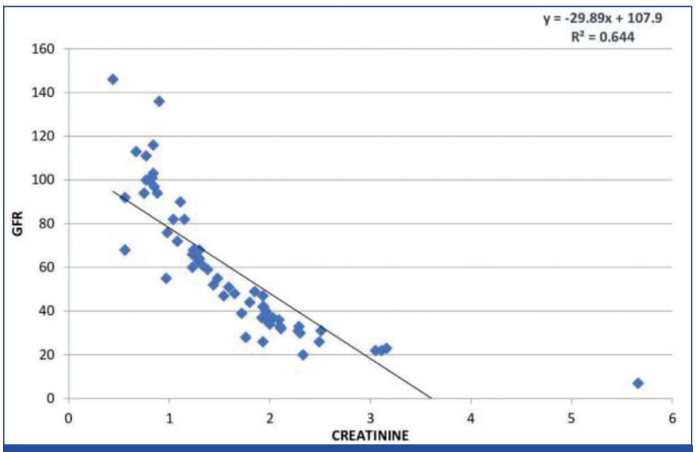
[Table/Fig-2]: Correlation between Creatinine and GFR and Cystatin C and GFR.

The overall mean GFR was calculated from both serum creatinine and serum cystatin C for the same groups of patients. GFR calculated from serum creatinine was found to be 59.57±31.48, higher than the GFR calculated from serum cystatin C, which was 31.25±18.32. Serum creatinine overestimates the GFR levels in comparison to serum cystatin C-based calculated GFR levels, which are significantly low (p<0.0001) [Table/Fig-3].

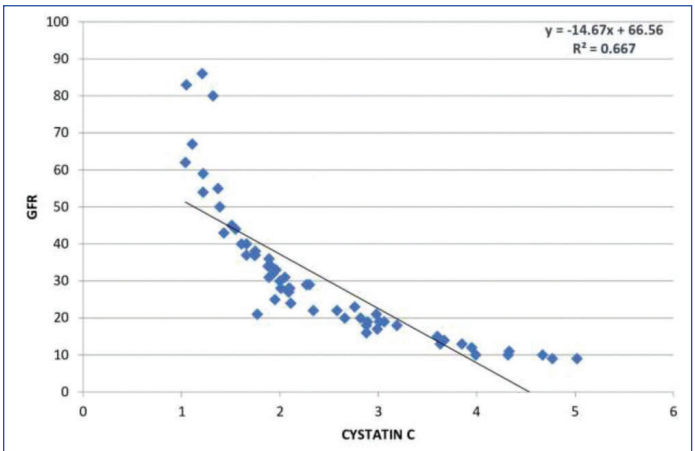
		p-value
GFR based on Creatinine	59.57±31.48	0.0001
GFR based on Cystatin C	31.25±18.32	

[Table/Fig-3]: Comparison of GFR measurement with Creatinine and Cystatin C.

In our regression analysis, the authors observed that for every unit increase in creatinine value [Table/Fig-4], there is a corresponding decrease in GFR by 29.89. Similarly, in [Table/Fig-5], the authors found that for every unit increase in cystatin C value, GFR decreases by 14.67. Also, there is a tighter distribution of results around the regression line for cystatin C indicated by R^2 value=0.67 versus 0.64 for creatinine.



[Table/Fig-4]: Linear regression plot between Serum Creatinine and GFR.



[Table/Fig-5]: Linear regression plot between serum Cystatin C and GFR.

DISCUSSION

Evaluation of renal function using serum creatinine-based assessments, such as creatinine clearance and e-GFR, can overestimate renal function, especially in patients with chronic liver disease and cirrhosis [17]. Several studies have reported that cystatin C is more useful and has a more significant correlation with GFR compared to serum creatinine in detecting moderate to severe renal impairment in patients with liver cirrhosis [18-22]. In patients with liver cirrhosis, cystatin C and cystatin C-based formulae or equations consistently showed a significant correlation to GFR and were measures that best discriminated early renal impairment, which aligns with the results seen in the current study. Given that patients with cirrhosis of the liver are extremely sensitive to even minor changes in GFR, which can have a significant impact on their survival, it is critical to identify markers that can detect renal impairment at an early stage [23].

Grubb A investigated the value of cystatin C in the evaluation of renal failure and concluded that cystatin C-based GFR-estimating equations are useful in all age groups, including the elderly, and outperform creatinine-based equations in predicting end-stage renal disease [24]. In a short follow-up study on patients with critical illness, Herget-Rosenthal S et al., discovered that serum cystatin C detected acute kidney injury 1-2 days earlier than serum creatinine estimation [25]. In a longer follow-up study, Seo YS et al., found that cystatin C was significantly higher in severe cirrhotic patients with normal creatinine who later developed HRS within one year, implying that cystatin C is a good predictor of HRS [26].

In his study, Sharawey MA et al., showed cystatin C as a predictor of HRS in patients with liver cirrhosis; a similar trend was noticed with mean serum creatinine at 1.04±0.8 and mean serum cystatin C at 1.8±0.8 [27]. A similar trend of cystatin C versus creatinine was also observed in the present study. Another study conducted by Kim DJ et al., also showed serum cystatin C as a useful marker for the evaluation of renal function in patients with decompensated cirrhosis (creatinine 0.8±0.2 versus cystatin C 1.1±0.3) [28].

Renal involvement is common in patients with liver cirrhosis due to the following factors: i) changes in haemodynamics; ii) activation of vasoconstricting hormones and neurohumoral systems such as the Renin-Angiotensin-Aldosterone System (RAAS), vasopressin, and endothelin; and iii) increased sympathetic nervous system activity. These haemodynamic alterations lead to a reduction in GFR, resulting in renal impairment and causing the retention of nitrogenous waste products. Additionally, the activation of the RAAS can cause sodium and water retention, leading to edema, which can further exacerbate renal dysfunction.

Typically, these changes in renal haemodynamics in liver cirrhosis are of a functional nature, which means that they are not accompanied by any structural or morphological changes and, in the early stages, can be reversed with lifestyle modifications and medical interventions. Randers E et al., discovered that serum cystatin C appeared to be a better parameter of GFR estimation than serum creatinine in adults with normal to moderately impaired kidney function in a study on serum cystatin C as an endogenous parameter of renal function [29]. Hojs R et al., concluded that the results of their study on serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of renal function indicate that serum cystatin C is a reliable marker of GFR and has a higher diagnostic accuracy than serum creatinine [30].

In the present study, it has been observed that serum creatinine and serum cystatin C can both be used for the estimation of GFR of the kidneys. When describing the stages of CKD according to the GFR values, cystatin C showed higher stages of CKD, which was reflected by lower GFR values in comparison to serum creatinine, which overestimated GFR values in the same group of patients. This is in accordance with the above-cited studies. It has been clearly concluded that serum cystatin C is superior to serum creatinine while calculating GFR values and accordingly staging CKD in patients.

Limitation(s)

In addition to its high cost, cystatin C is not standardised and can be influenced by other factors such as infections, chronic metabolic diseases like diabetes and thyroid disease, as well as those with elevated levels of inflammatory markers and those treated with steroids. The current study did not include any inulin-based or radio-labeled Ethylene Diamine Tetra-Acetic Acid (EDTA) studies, which are considered gold standards for measuring GFR.

CONCLUSION(S)

The Cystatin C levels are significantly higher in patients with liver cirrhosis who develop renal dysfunction. An elevated level of cystatin C can indicate a decrease in GFR even before the serum creatinine level increases. Therefore, measuring the serum cystatin C level can be a useful tool for the early detection and monitoring of renal dysfunction in patients with liver cirrhosis. Further research with larger prospective cohort studies is required to address this important gap in clinicians understanding of this issue.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: May 18, 2023
- Manual Googling: Dec 25, 2023
- iThenticate Software: Dec 27, 2023 (15%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 15, 2023**
Date of Peer Review: **Aug 04, 2023**
Date of Acceptance: **Dec 30, 2023**
Date of Publishing: **Mar 01, 2024**