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Comparative study of serum cystatin C and urinary creatinine in acute kidney failure patients

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Abstract

Background: Acute kidney failure (AKF), or acute kidney injury (AKI), is a rapid decline in renal function, typically defined by an increase in serum creatinine, a decrease in urine output, or both. AKF can result from a variety of causes, including prerenal, renal, and postrenal factors, and is commonly seen in critically ill patients in intensive care units (ICUs) or those undergoing major surgeries.

Material and methods: Comparative study. Individuals that come to Padmashree diagnostic center; Vijayanagar for checkup will be recruited for the study after explanation of the study to the individual; a written informed consent was obtained. Total of 60(n=60) urine and blood samples from healthy individuals(n=30) and CKD patients(n=30)were collected and recruited for the study. The study was approved by institutional review board.

Result and discussion: The present study was carried out to assess the status of Cystatin C and Urinary Creatinine levels in renal failure patients. The patients were recruited for the study at Padmashree diagnostics, Department of Clinical laboratory, Vijayanagar, Bangalore. The study initiation started after obtaining the written informed consent.The study population consisted of 30 participants of normal individuals (n=30) and acute renal failure subjects (n=30), mean age of 38.11 ± 4.3 and 54.40 ± 4.6 respectively is suspected to be suffering from CKD.

Conclusion: A cross sectional case control study was conducted. It consists a total of 60 subjects(n=60). 30 healthy individuals – Having normal serum creatinine level and 30 Renal failure patients – Having serum Creatinine level >4.0 mg/dl. The subjects were recruited at Padmashree diagnostics, Vijayanagar, Bangalore.

Keywords: Urine; Creatinine; Cystatin; CKD; Jaffe's method

1. Introduction

Acute kidney failure (AKF), also referred to as acute kidney injury (AKI), is a clinical syndrome characterized by a sudden decline in renal function, resulting in the accumulation of waste products, disturbances in fluid and electrolyte

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balance and a risk for life-threatening complications. AKF is a common and serious medical condition that can occur in a variety of clinical settings, such as sepsis, dehydration, trauma, nephrotoxic drug exposure, or acute glomerulonephritis. It is often diagnosed through an increase in serum creatinine (SCr) levels and a decrease in urine output. However, these traditional markers of renal dysfunction can sometimes be delayed or inadequate for detecting early-stage kidney injury.

Given the global burden of AKF and the challenges in early diagnosis and timely intervention, identifying reliable biomarkers could substantially refine clinical practices and aid in the development of more personalized treatment strategies for AKF patients.

1.1. Kidney

The Urinary System is composed of a pair of kidney and urinary tract. Kidney filters the blood removing the metabolic wastes in body maintaining fluid; acid base balance; electrolyte and blood pressure homeostasis. With this Urine is produced which is eliminated by urinary system. The kidney has different types of function; excretory functions; regulatory functions; endocrine functions.

- **Excretory Functions:**Excretion of most of the undesirable end product of metabolism. Excretion of any excess of inorganic substances ingested in diet. Excretion of the waste products including NPN, Inorganic acids e.g., amino acids.
- **Regulatory Functions:** Mechanisms of differential reabsorption and secretion, this operates under complex system of control.
- **Endocrine Functions:** Primary- Because the kidneys are endocrine organs producing hormones e/g prostaglandins
  Secondary-because the kidneys are the site for hormones produced or activated elsewhere.[2]

In the early stages of chronic kidney disease, you may have few signs or symptoms. Chronic kidney disease may not become apparent until your kidney function is significantly impaired.[3]Treatment for chronic kidney disease focuses on slowing the progression of the kidney damage, usually by controlling the underlying cause. Chronic kidney disease can progress to end-stage kidney failure, which is fatal without artificial filtering (dialysis) or a kidney transplant.[4] Signs and symptoms of chronic kidney disease develop over time if kidney damage progresses slowly. Signs and symptoms of kidney disease may include:[5] Nausea,VomitingLoss of appetite,Fatigue and weakness,Sleep problems,Changes in how much you urinate,Decreased mental sharpness,Muscle twitches and cramps,Swelling of feet and ankles,Persistent itching,Chest pain, if fluid builds up around the lining of the heart,Shortness of breath, if fluid builds up in the lungs,High blood pressure (hypertension) that's difficult to control.Signs and symptoms of kidney disease are often nonspecific, meaning they can also be caused by other illnesses. Because your kidneys are highly adaptable and able to compensate for lost function, signs and symptoms may not appear until irreversible damage has occurred.[6]

Causes of Chronic kidney disease occurs when a disease or condition impairs kidney function, causing kidney damage to worsen over several months or years.[7]

Diseases and conditions that cause chronic kidney disease include:[8]Type 1 or type 2 diabetes,High blood pressure, Glomerulonephritis,an inflammation of the kidney's filtering units (glomeruli),Interstitial nephritis, an inflammation of the kidney's tubules and surrounding structures,Polycystic kidney disease,Prolonged obstruction of the urinary tract, from conditions such as enlarged prostate, kidney stones and some cancers,

1.2. Complications

Chronic kidney disease can affect almost every part of your body. Potential complications may include:[9] Fluid retention, which could lead to swelling in your arms and legs, high blood pressure, pulmonary edema,hyperkalemia, which could impair your heart's ability to function and may be life-threatening,cardiovascular disease,weak bones and an increased risk of bone fractures,Anemia.

1.2.1. Kidney Function Tests:[10]

- **Renal Clearance and Glomerular Filtration Rate** Clearance= U×V/P ml/minute V= Urine flow rate ml/minute P=plasma concentration of substance U= Urine concentration of substance

1.2.2. Assessment of glomerular permeability.

- **Measurement of non-protein nitrogenous compounds(NPN)**

e.g. - Creatinine, Uric Acid, Ammonia and Amino Acids.

1.3. Creatine and Creatinine

1.3.1. Creatine: [11]

- Creatine is synthesized in liver, kidneys and pancreas by two enzymatically mediated reactions:
- Transaminationof arginine and glycine to give guanidoacetic acid.
- Methylation guanidoacetic acid in presence of S- adenosyl methionine as methyl donor to give creatine.

1.3.2. Fate of Creatine

- Creatine is transported into site of usage mainly muscles and brain.
- About 1-2% of the total muscle Creatine pool is converted daily to creatinine through the non-enzymatic loss of water.
- Creatine in muscle will be phosphorylated to give creatine phosphate (High Energy Compound).

1.3.3. Creatinine:[12]

Creatinine (Cr) is an important Bio marker for kidney Function. Creatinine is an anhydride of creatinine and a breakdown product of creatinine phosphate in muscle. The production of Cr. in body is normally constant depending upon the muscle mass and is not influenced by metabolism or dietary factors. Cr is a waste product that is produced at the end of creatinine metabolism. Creatinine is filtered at the glomeruli and is secreted by the tubules and is excreted via urine per 24 hours in 1.5-3.0gm. The level of creatinine increases in certain conditions mainly in renal diseases.

1.3.4. Excretion of Creatinine:

Creatinine in plasma is filtered freely unchanged at the glomerulus. A small amount of it undergoes the tubular reabsorption. Up to 7-10% of urinary creatinine results from tubular secretion, therefore the glomerulus filtration rate (GFR) was most often assessed by determining the urinary creatinine clearance.

Clinical Significance of Creatinine and Creatinine Clearance:[13]

Creatinine clearance may be used as indicator for GFR because:

- Creatinine is endogenously produced.
- Creatinine is released into body fluid at constant rate.
- Its plasma level maintained within narrow limits.
- Its plasma level not affected by dietary factors.

1.4. Cystatin C:[14-20]

Cystatin C (Cys C) is an endogenous cysteine proteinase inhibitor belonging to the type 2 Cystatin superfamily. The mature, active form of human Cystatin C is a single, low-molecular-weight (MW 13360), non-glycocylated cationic basic protein consisting of 120 amino acids and belongs to the Cystatin superfamily of endogenous Cysteine proteinase inhibitors. Although serum creatinine concentration is the most commonly used indicator of kidney function, it is not an accurate marker of glomerular filtration rate (GFR), which is an important index of renal function in health and disease. On the other hand, the plasma concentration of CysC has been shown to be a better marker of GFR in both adults and children. CysC possesses most of the properties of an ideal GFR test - produced by all nucleated cells at a constant rate, it is freely filtered from the glomerulus and finally, is fully destroyed in the proximal renal tubules.

1.4.1. Cystatin C and low-molecular-weightproteins

Cystatin C is not the potential replacement for creatinine as a marker of GFR; several so-called lowmolecular- weight proteins have been considered, including a-1 microglobulin, retinol-binding proteinand b-2 microglobulin.Whilst automatedimmunoassay techniques are available for these proteins which are free from interference frombilirubin and hemoglobin and are at least potentially adaptable to automated analysis, all of these markerhavebeendiscarded due to failure tomeetoneormore of the above GFRmarker criteria. Ingeneral, this has beenbecause their production rates are not constant and because theirlimitation fromplasma is not entirely bythe renal route. However, it was whilst exploring the inter-relationship of low-molecular-weight proteinswith GFR that the relationship of Cystatin C with decreasing GFRwas demonstrated in1985.[45]

1.4.2. Physiological function of Cystatin C

Cystatin C is a member of the Cystatin superfamily of cystine protease inhibitors. There are now 11members of this family and Cystatin C is considered the most physiologically important inhibitor ofendogenous cystine proteases.32 These include the cathepsins B, H and L, and the role of Cystatin C isbelieved to be the regulation of such proteases secreted or leaked from the lysosomes of dying or diseased90 Newman *Ann Clin Biochem* 2002; **39**: Cystatin C 91 cells. The cathepsins are also important in extracellular matrix degradation, and Cystatin C has been shown to decrease the invasiveness of melanomas, a process that is dependent upon the degradation of extracellular matrix proteins Cystatin C has also been shown to increase the proliferation of mouse broblasts and rat mesangial cells, cell types that are also involved in extracellular matrix production and breakdown. Recently, there is evidence for inhibitionof osteoclastic bone resorption, which is yet another process involving extracellular matrix breakdown.

1.4.3. Structure of cystatin C

Cystatin C is a 13359 Da protein, is non-glycosylated and contains two disulphide bridges. It consists of 120 amino acids but is synthesized as a preprotein (indicating an extracellular function) and is the product of a 7¢3-K base gene found on chromosome [46] The native protein has a pI of approximately9¢3, but an additional form has been isolated from urine having a lower pI of 7¢8.44

1.4.4. Synthesis and secretion of cystatin C

Using both immunocytochemistry and *in situ* hybridization, Cystatin C has been identified in a wide range oforgans and cell types (*see* Table 1). Cystatin C has also been measured in a range of biological fluids, asshown in Table 2. One of the key criteria that Cystatin C needs to meet to be a potential replacement for creatinine is that its production rate should be constant or at least less variable than that of creatinine. There is good evidence that all nucleated cells constitutively express Cystatin C.[39] For example, the promoterregion of the Cystatin C gene has several of the features of a housekeeping gene, that is, it has no CAAT box,has multiple GGGCGG regions and has an AT-rich sequence in the transcription regulation region.42

There are some deference's fromclassical housekeeping genes however. For instance, there is a GATAAA sitethat is analogous with the transcription factor binding site 2D, and there are also now recognized to be bindingsites for the transcription factors AP-2 andMEP1.42 It is now clear from a variety of *in vitro* studies thatsome stimuli increase Cystatin C production.[39] For instance, dexamethasone has been shown to cause adose-dependent increase in Cystatin C production by more than 50% in HeLa cells.[39] Exposure of mouseembryo cells to transforming growth factor-b (TGF-b), a pro-biogenic growth factor, increases Cystatin Cmessenger RNA production, and exposure of alveolar macrophages to arsenic oxide can also cause anincrease in production of Cystatin C. By contrast, activation of monocytes and macrophages with lipopolysaccharide can cause a decrease in the expression of Cystatin C.

Whilst this provides some evidence for the deferentialregulation of Cystatin C expression in different tissues, which does not appear to be as a result ofaltered messenger RNA stability, little else is clear.41There is, therefore, evidence suggesting that the synthesis of Cystatin C is not constant, but there havebeen no studies that demonstrate any major influence 0f exogenous agents, infection or malignancy oncirculating serum concentrations of Cystatin C.Intuitively, if all nucleated cells produce Cystatin C, then a large tumor burden should result in anincrease in production. Whilst some studies showed increased Cystatin C production by more aggressivetumors, there has been debate as to whether any alterations in the underlying GFR have beenaccounted for. A recent publication by the group that raised this concern56 demonstrated that if theGFR is measured by a reference technique then Cystatin C concentrations were unrelated to tumor burden. Nevertheless, if it is established that tumors*Ann Clin Biochem* 2002; [46]

1.4.5. Clearance of cystatin C

Only one published study has evaluated the clearance of Cystatin C, using iodinated protein in rats. Theclearance of Cystatin C was approximately 94% of that of chromium EDTA and the extra renalclearance wasless than 0¢34mL/min, suggesting that elimination from the circulation was almost entirely throughglomerular ¢alteration. Cystatin C is also metabolized, or at least cleaved, by the protease papain (or possiblyby a contaminant of papain preparation, papaya protease IV; Soren Blirup-Jensen, DAKOA/S, personalcommunication) and by neutrophil elastases.

In some rare forms of amyloidosis, Cystatin C has beenshown to dimerize and form amyloid broils. It is also clear that Cystatin C is metabolized by the proximaltubular epithelial cells, as indeed are all proteins altered by the glomerulus. Thus, once ¢altered,Cystatin C will not return to the circulation in an intact form, although it may return after degradationinto smaller peptides and/or their constituent amino acids. Several authors have attempted to look for tubular secretion of low-molecular-weight proteins such as cystatin C[46].

2. Review of literature

Abnormalities of body composition and impaired nutritional status are common findings with in every stages of Chronic Kidney Disease (CKD). [21] CKD is a common and serious complication associated with increased risk of mortality, progression to kidney failure, cardiovascular disease (CVD) and hospitalizations. Accurate estimation of glomerular filtration rate (GFR) is essential for the diagnosis, staging, and management of CKD. [25,26]

Urine Creatinine being widely used as a marker of CKD, in the detection of abnormalities in Renal Function and Dysfunction of Kidneys.[22]Urine creatinine (UCr) excretion is easy available and reliable approximation of muscle mass in healthy individual and individuals with CKD.[22,24] However, because extra renal excretion increasingly contributes to the Total Creatinine excretion as renal function declines, there is uncertainty as to whether UCr predicts clinical outcomes independently of kidney function.[23]In some patients with kidney failure who are being treated under hemodialysis or peritoneal dialysis with some degree of residual kidney function, High UCr is associated with better outcomes.

Creatinine is the stable end product of creatine. Most creatine is present in muscle and is converted at a steady rate to creatinine. Creatinine is released into the circulation and is almost exclusively excreted in the urine. [36]In steady state conditions, urinary excretion will equal creatinine production, irrespective of the serum creatinine concentration. Therefore, measurement of urinary creatinine excretion (UCE) in 24-h urine collections is a widely accepted method for muscle mass estimation in stable outpatient populations.[37, 38, 39] In healthy subjects [40] and in patients with renal failure,[41, 42]UCE has been associated with long-term mortality.

Renal outcomes after critical illness are seldom assessed despite strong correlation between chronic kidney disease and survival. Renal dysfunction is more strongly associated with mortality when assessed by serum Cystatin C than by creatinine.[43]

Cystatin-C is the low-molecular-weight cysteine protease inhibitor, Cystatin C is synthesized in all nucleated cells at a constant rate and exhibits favourable properties for a renal biomarker such as free filtration by the glomerulus with no reabsorption into the blood. Compared to creatinine, Cystatin C is less influenced by diet, muscle mass, or body constitution, making it a reliable marker of the glomerular filtration rate (GFR) and hence renal function. [27-32]

The mature, active form of human Cystatin C is a single non-glycosylated polypeptide chain with a molecular radius of 1.51 nm, containing 120 amino acid residues preferentially abundant in CSF, seminal plasma, milk, synovial fluid, saliva, tears, urine, and blood plasma. No method permitting the measurement of Cystatin C in all of these human body fluids was available until 1979 when two suitable enzyme amplified immunoassays were introduced. [33] Since then, levels of Cystatin C in various body fluids have been used as markers of various diseases.

The concentration of plasma (serum) Cystatin C in healthy individuals ranges around 0.8–1.2 mg/l, depending on analytical methods. In babies, plasma levels are significantly higher, varying with age in the first year of life. Change in the serum concentration of Cystatin C has been proposed as an index of kidney function, increased serum levels are almost exclusively associated with a reduction in GFR. Storage of serum samples at various conditions does not significantly affect Cystatin C. Previous reports demonstrated that the protein is stable for at least six months when plasma/serum samples are frozen at −80 ◦ C. Finally, blood can be left unseparated up to 24hours without adverse effect. [34]

Physiologically, In Kidney the proximal tubular cells have the ability to reabsorb and catabolize all of the freely filtered Cystatin C, thus, the normal urinary concentration of Cystatin C is very low, ranging from 0.03 to 0.30 mg/l, as first found by Lofberg and Grubb in 1979. [33]

The concentration of serum Cystatin-C (Cys-C) is correlated with creatinine (Cr), and is mainly determined by glomerular filtration; thus, Cys-C may be an index of the Glomerular filtration rate (GFR).However, the excretion of serum Cys-C and Cr excretions are unclear in healthy individuals and CKD patients. [35]

Thus, in this study we investigated the difference of serum Cys-C and Cr excretions, and examined whether the serum Cys-C or UCr is a better marker for CKD.

2.1. Aims and objective of study

Estimation of urinary creatinine level inhealthy individuals and Renal dysfunction patients.

Correlating the Cystatin C values in healthy individuals and Renal dysfunction patients.

3. Material and methods

Individuals that come to Padmashree diagnostic center; Vijayanagar for checkup will be recruited for the study after explanation of the study to the individual; a written informed consent was obtained. Total of 60(n=60) urine and blood samples from healthy individuals(n=30) and CKD patients(n=30)were collected and recruited for the study. The study was approved by institutional review board.

- **Study Design:**Case Control Observational Study
- **Sample Size:** Total of 60 urine samples(control -30 and case -30)

3.1. Methods of collection of data

3.1.1. Inclusion Criteria

- Sample should be collected fromPatient with moderate Kidney Diseases and healthy individuals of both sexes.
- Patient with acute Kidney Diseases. Age range between 25-60 years.
- The subjects whose serum creatinine values were more than4mg/Dl

3.1.2. Exclusion Criteria

- Patient with any clinical history of any other disease than acute Kidney Diseases.
- Age above 25 years and below60 years.
- Sample should not contain any typeof anticoagulant.
- Pregnant ladies not required for the studies.
- Hypertension subjects are also not required.
- The study was submitted and approved by institutional review board.

3.2. Method

3.2.1. Latex enhanced immunoturbidometric method

The Cystatin C test system is a device intended for the invitro quantitative determination of Cystatin C in serum or plasma.

3.2.2. Assay principle:

Sample is reacted with a buffer and anti-CystatinC coated latex. The formation of the antigen antibody complex during the reaction results in an increase in turbidity. The extent of which is measured as the amount of light absorbed at 570 nm. By constructing a standard curve from the absorbance of the standards, Cystatin C concentration of sample is determined.

3.2.3. Source of Data

Available literature information from recent publication will be updated during the course of study. The analytical technique to be employed as indicated will be standardized/ modified depending upon the situation before applying the same for the sample analysis. Information with respect to the clinical diagnosis will proceed from the normal individual and the clinical expertise opinion will be sought before the study outcome.

3.3. Statistical Analysis

SPSS Ver19 Data analysis package was used and applied to analyze the obtained data after discussion with the Biostatistician. All the values were expressed in mean ± SD. Statistical comparison was performed using student t test. The student's' t' test *p< 0.05; **p< 0.01; ***p< 0.001 was considered as significant**.**

3.4. Assay protocols

The analytes of interest in serum were assayed by the following methods. In general, the methods followed are described in detail with respect to the principle of the assay methods, nature of reagents, reference materials (Calibrators) and their source, instruments used and limitation of the assay methods if any with appropriate references, in the following sections.

Note

- The source of reagents is specifically stated.
- Details regarding the assay methods with respect to the precise volumes of specimens / reagents and assay conditions etc., is provided below

Table 1 Analytical method

Contents	**Concentration of solutions**
Reagent 1	Tris-buffer solution.
Reagent 2	Suspension of latex particles coated with rabbit anti human cystatin c polyclonal antibody.

3.4.1. Stability and preparation of reagents:

- All reagents are ready to use.
- Stable up to the expiry date when stored at 2-8^{o}C

3.4.2. Assay procedure

Test procedure

- Assay mode: 2-point rate 19-34
- Wavelength (main/sub): 570nm/800nm

3.4.3. Method

Table 2 Reagent leaflet protocol

	RB	**S1**	**S2**	**S3**	**S4**	**S5**	**C1**	**C2**	**T1**
Tris buffer R1(µl)	-	180	180	180	180	180	180	180	180
D/W (µl)	183	-	-	-	-	-	-	-	-
Std (µl)	-	3	3	3	3	3	3	3	3
Latex reagent/R2 µl(µl)	60	60	60	60	60	60	60	60	60

Incubate at 30 seconds at 37 ºc.

Read the value after 10 minutes at 570nm.

Calibration: Recommended that this assay should be calibrated using Gcell calibrator (Cat.no.GC-CystC-L)

3.4.4. Calculation:

By constructing a standard curve from the absorbance of the standard, Cys-C concentration of sample can be determined. Do not attempt to extrapolate above or below the range of the calibrators.

Quality control: Commercial QC sera was used.

Table 3 The concentration of substances in reagent

Controls	**Concentration (mg/L)**
C1 (Normal control)	0.85 ± 0.13
C2 (Abnormal c ontrol)	1.70 ± 0.25

3.4.5. Biological reference intervals

- Age: 1- 60 years : < 1.03mg/L

- Age:> 60 years : <1.26mg/L

3.4.6. Specific performance characteristics

Linearity

The method is linear up to 8.0mg/l sample above this concentration should be diluted with 0.9% NaCl and repeat assay. Multiply the result by diluting factor.

Creatinine

Method: Jaffe's method (Kinetic Method):

Principle:

The kinetic colorimetric assay is based on the jaffe method.In alkaline solution, creatinine forms a yellow-red complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen.

Creatinine + Picric acid $\xrightarrow{\text{Alkaline pH}}$ Yellow-red complex

Reagent-working solutions

Component	concentration		
	R1	R2= SR Test	
Potassium hydroxide	900	80	mmol/L
Phosphate	135	12	mmol/L
Picric acid	38	4.4	mmol/L
PH	≥13.5	6.5	13

Reagent R2 contains a nonreactive buffer

3.4.7. Specimen Collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers. Collect urine without using additives. If urine must be collected with a preservative for other analytes, only hydrocholoric acid (14 to 47 mmol/l urine e.g., 5ml 10% HCL or 5ml 30% HCL per litre urine). Or boric acid (81 mmol/l e.g., 5gm per litre urine) may be used.

Urine samples are automatically prediluted 1:25 (1+24) with water by the instrument.

Stability without preservative:

- 2 days at 15 -25 ^{0}C
- : 6 days at 2-8 ^{0}C
- : 6 months at -15 to -25 ^{0}C

Stability with preservative

- : 3 days at 15 -25 ^{0}C
- : 8 days at 2-8 ^{0}C
- : 3 weeks at -15 to -25 ^{0}C

Centrifuge sample containing precipitate before performing the assay.

3.4.8. Assay

For optimum performance of the assay follow the direction given in the document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer specific assay instructions.

3.4.9. Applications for Urine

Table 4 COBAS INTEGRS 400/400 plus test definition test instrument (Table showing standard operating protocol of instrument)

Measuring mode	**Absorbance**
Abs. calculation mode	Kinetic
Reaction direction	Increase
Wavelength A/B	512/583nm
Calc first/last	40/49
Reaction mode	D-R1-S-SR
Predilution factor	25
Unit	mmol/L

Table 5 Pipetting parameter of reagent and sample according to the standard operating parameter of instrument.

		Diluent (H_2O)
R1	13 µL	71µL
Sample	10 µL	20 µL
SR	17 µL	16 µL
Total volume	147 µL	

Table 6 Calibration

Calibrator	Calibrator F.A.S use deionized water as zero calibrator.
Calibrator mode	Linear regressions
Calibrator replicate	Duplicate recommended.
Calibration interval	Cobas integra 400/400 plus analyzer: each cobas C pack, every 7 days, and as required following quality control procedures.

Table 7 Quality control Table showing Quality control of reagent

Reference range	**Precinorm PUC**
Pathological range	Precipath PUC
Control interval	24hr recommended
Control sequence	User defined
Control after calibration	Recommended

- For quality control use control materials as listed in the "order information" section. Other suitable control materials can be used in addition.
- The control intervals and limits should be adapt to each laboratory's individual requirements. Value obtained should fall within the defined limits.
- Each laboratory should establish corrective measures to be taken if value fall outside the limits.
- Follow the applicable government regulation and local guidelines for quality controls.

Calculations

Cobas integra analyzer automatically calculate the analyte concentration of each samples.

Conversion factor: mmol/L × 11.3= mg/dl

Limitations:

Criterion recovery in the creatinine decision range for adults (20mmol/l in urine) within ± 10% of initial value.

Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability, intermediate precision (two aliquots per run, 2 runs per day, 20 days).

The following results were obtained:

Table 8 Precision of reagent

	Level 1	**Level 2**
Mean	2.16mmol/l (24.4 mg/dl)	19.1mmol/l (216mg/dl)
CV repeatability	1.4%	0.81%
CV intermediate precision	2.5%	1.6%

4. Results and discussion

The present study was carried out to assess the status of Cystatin C and Urinary Creatinine levels in renal failure patients. The patients were recruited for the study at Padmashree diagnostics, Department of Clinical laboratory, Vijayanagar, Bangalore. The study initiation started after obtaining the written informed consent.The study population consisted of 30 participants of normal individuals (n=30) and acute renal failure subjects (n=30), mean age of 38.11 ± 4.3 and 54.40 ± 4.6 respectively is suspected to be suffering from CKD.

The healthy individuals with normal levels of creatinine were used as control samples for this study. It is apparent that Cystatin C elevation is one of the common complications in kidney failure patients. Accordingly, with an emphasis on the required sensitivity of the assay methods, the present study was carried out on Cystatin C by a validated available analytical method for serum analysis that is often used in the laboratory medicine.

4.1. Urinary creatinine study:

As a part of the study, the levels of urinary creatinine were studied among the healthy population and Renal failure patients. Levels of urinary creatinine in the control group was 50.22 ± 34.65 mg/dl and in the Test group / Renal failure patients' group was 13.0 ± 3.95. It is found that Urinary creatinine levels were significantly lower in renal failure patients ($p = < 1.0$), compare to healthy population (Fig.5.1)

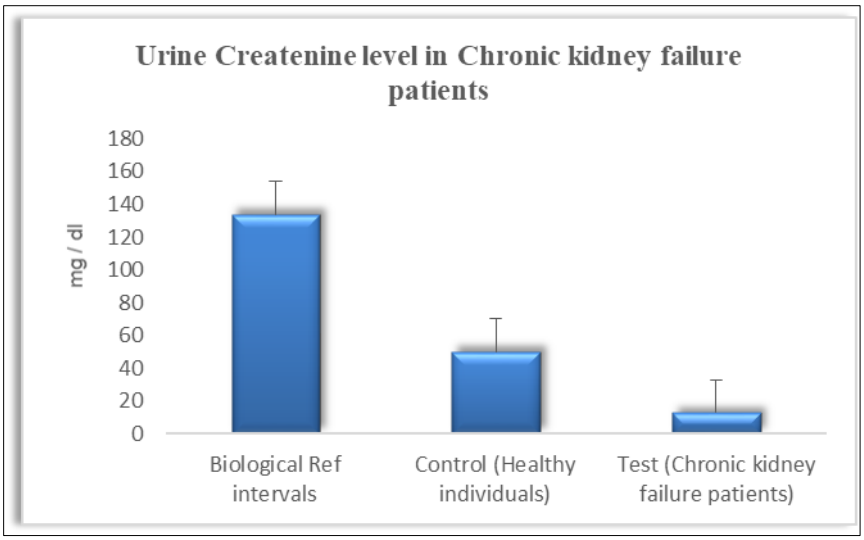

Figure 1 Histogram representing the levels of urinary creatinine in healthy population and Renal failure patients

4.2. Serum Cystatin C study

Levels of Cystatin C (a marker of renal failure) was also studied in the same two groups. Levels of Cystatin C in healthy population was 1.02 ± 0.3 mg/L and in Renal failure patients it was 4.3 ± 0.34 mg/L.It is observed that levels of Cystatin C is significantly higher (p = < 1.0) in Renal failure patients compare to Healthy individuals. (Fig 5.2)

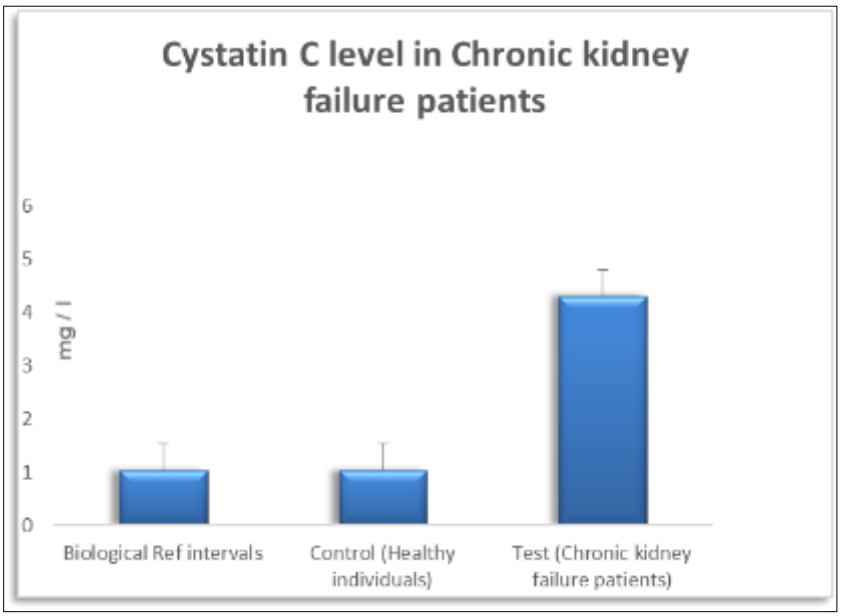

Figure 5.2 Histogram representing the levels of serum Cystatin C in healthy population and Renal failure patients

5. Conclusion

A cross sectional case control study was conducted. It consists a total of 60 subjects(n=60). 30 healthy individuals – Having normal serum creatinine level and 30 Renal failure patients – Having serum Creatinine level >4.0 mg/dl. The subjects were recruited at Padmashree diagnostics, Vijayanagar, Bangalore.

Creatinine levels and Cystatin C were estimated in the random urine and Serum samples respectively. Our study showed that theurinary creatinine levels were significantly lower in renal failure patients (p = < 1.0), compare to healthy population (Fig.5.1). It may be due to the decreased Creatinine clearance by kidneys. Cystatin C is significantly higher (p = < 1.0) in Renal failure patients compare to Healthy individuals. (Fig 5.2).

The study concludes that Urinary creatinine and Serum Cystatin C levels are inversely correlated in renal failure patients.

In routine clinical practice Cystatin C should also be included as a kidney function test along with creatinine clearance test.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interests.

Author Contributions

Concept and design: AT,SB,BPM and JBO; Statistical analysis: ATM,SB,PM and DBP; Writing of the manuscript: AT,SK ,SA and PT; Data collection: AT and SB; Revision and editing AT,SB, JBO, and SA . All authors have read and agreed with the contents of the final manuscript towards publication

Statement of informed consent

Informed consent was obtained from all individual participants included in the study. who visited Padmashree Diagnostic Center, Vijayanagar, Bangalore.

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