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Serum and urinary cystatin C as early biomarkers for renal dysfunction in dogs

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Abstract
Renal dysfunction in dogs, encompassing both acute kidney injury (AKI) and chronic kidney disease (CKD), poses diagnostic challenges due to limitations in conventional biomarkers like serum creatinine and blood urea nitrogen (BUN). This study evaluated the diagnostic utility of serum and urinary cystatin C (Cys-C) in differentiating renal dysfunction stages in dogs. Thirty-seven dogs were enrolled, including six healthy controls, six with AKI, and twenty-five with CKD stratified by IRIS staging. Serum and urinary Cys-C concentrations were significantly elevated in dogs with renal dysfunction compared to controls, with progressive increases observed across CKD stages. Notably, Cys-C levels were less influenced by age, sex, or muscle mass, enhancing its reliability as a biomarker. The findings support Cys-C as a sensitive and early indicator of renal impairment, with potential to improve diagnostic accuracy and staging in canine nephrology. Incorporation of Cys-C into routine diagnostic panels may facilitate timely intervention and better clinical outcomes.

Keywords: Cystatin C, acute kidney injury, chronic kidney disease, biomarkers, IRIS staging, serum creatinine, blood urea nitrogen, dogs.

1. Introduction
Renal dysfunction means structural or functional damage in one or both kidneys [9]. AKI is typically marked by a sudden decline in glomerular filtration rate (GFR), reduced urine output and impaired renal solute excretion [4]. In contrast, CKD is characterized by long-standing functional or structural abnormalities in one or both kidneys, usually persisting for two months or more. Both conditions can coexist in the same patient, a situation referred to as acute on chronic kidney disease. Generally, CKD is considered irreversible and progressive, while AKI may be reversible with timely intervention [25]. In canine veterinary medicine, chronic kidney disease (CKD) is staged following the International Renal Interest Society (IRIS) criteria, which incorporate sub-staging parameters such as systemic blood pressure and urinary protein levels to assess disease severity and predict clinical outcomes [2, 25]. Diagnostic frameworks for acute kidney injury (AKI) and chronic kidney disease (CKD) are instrumental in recognizing instances of renal damage that may be amenable to therapeutic intervention. Nonetheless, accurate classification of the injury type and underlying pathology remains a critical first step for the veterinarian [6]. Blood urea nitrogen (BUN) and serum creatinine are principal metabolic byproducts routinely eliminated by the kidneys. When renal function is compromised, these substances accumulate in the bloodstream, making their elevated concentrations reliable biochemical markers for detecting kidney dysfunction [14]. At 75-90% loss of kidney function, waste builds up in the blood, causing symptoms like poor appetite, weight loss, lethargy, vomiting, and diarrhoea [26, 27]. On the other hand, identifying reliable early CKD biomarkers remains a challenge.
Emerging urinary biomarkers such as low molecular weight proteins and tubular enzymes may act as sensitive early indicators of renal injury or impairment, preceding detectable alterations in glomerular filtration rate (GFR) [7, 12]. These markers also offer compartment-specific insights into renal involvement and could facilitate the early identification of tubulointerstitial

nephropathies, even in the absence of proteinuria [7, 22]. Serum cystatin C is consistently produced by all nucleated cells and eliminated via glomerular filtration in both humans and dogs [1]. Since Cys-C is low molecular weight protein it is freely filtered by the glomerulus and does not undergo secretion by tubular cells [11]. Filtered cystatin C is reabsorbed through megalin-facilitated endocytosis in the proximal tubules before undergoing catabolism [16]. Whenever there is proximal tubular injury or dysfunction, this reabsorption and degradation is impaired, leading to increased urinary cystatin C (uCysC) levels [8].

This investigation evaluated the diagnostic potential of serum and urinary cystatin C for early detection of Renal dysfunction in dogs, and explored its correlation with established renal biomarkers including blood urea nitrogen (BUN) and serum creatinine (CREA).

Materials and Methods

The present study was carried out in the Veterinary College Hospital, Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru, KVAFSU University Bidar.

Animals for the study

Apparently healthy (control) dogs

Six apparently healthy dogs irrespective of breed and gender with age more than 6 years brought to the Veterinary College Hospital, Hebbal, Bengaluru for regular health examination were considered as the control group. Animal with any medication was not included.

Clinical cases (Study group)

Dogs aged above six years presented to the Veterinary College Hospital, Hebbal, Bengaluru with clinical signs indicative of renal dysfunction were selected as study population.

AKI Group

Dogs aged above six years presented to the Veterinary College Hospital, Hebbal, Bengaluru with history, clinical signs, laboratory findings and renal sonography changes suggestive of acute kidney injury were considered as AKI Group.

CKD Group and Staging of CKD

Dogs aged above six years presented to the Veterinary College Hospital, Hebbal, Bengaluru with history, clinical signs, laboratory findings and renal sonography changes suggestive of chronic kidney disease were considered as CKD Group.

Staging of CKD was done based on the guidelines of IRIS board as Stage I (<1.4 mg/dL), Stage II (1.4-2.8 mg/dL), Stage III (2.9-5.0 mg/dL) and Stage IV (>5.0 mg/dL). The IRIS stage of CKD was determined according to the 2019 version, based on serum creatinine concentration. To stage CKD based on serum creatinine values minimum of two serum creatinine values were taken at week apart to assess the stability of CKD.

Collection of Clinical samples

Serum

Two millilitres of whole blood was collected from cephalic or saphenous vein of both suspected and apparently healthy dogs in AV LaboTube serum vacutainer and allowed for stable clot formation at room temperature for 20 min, and then the samples were centrifuged at 3000 rpm for 10 min to collect the serum samples and processed the sample using semi-automatic serum biochemical analyzer RX-50 of Micro Lab for estimation of serum creatinine and blood urea nitrogen. The remaining serum sample was stored at-80ºC for serum cystatin C estimation.

Urine

About 10 millilitres of urine were aseptically collected from both apparently healthy and clinically suspected dogs, using sterile urine collection vials preferably via catheterization to minimize contamination. The sample was stored at-80ºC for urine cystatin C estimation.

Methods

Serum cystatin C estimation

The Canine Cystatin C ELISA kit with catalog number E0148Ca made by Bioassay Technology Laboratory was used for serum cystatin C estimation.

Urine cystatin C estimation

Cystatin C ELISA Kit with catalog number LAB0335 made by LabReCon, was procured from Universal Biotechnology Pvt Ltd and used for urine cystatin C estimation.

Statistical Analyses

The numerical data obtained in this study from both apparently healthy dogs and the dogs with Renal dysfunction were examined using statistical techniques including Mean, Standard Deviation, Standard Error, and independent T-Test for evaluating the significance of comparison.

Results and Discussion

Study population

The study include total of 6 apparently animals and 31 dogs with Renal dysfunction. Out of 31 dogs 6 dogs were each of AKI, stage 1, stage 2 and stage 3 CKD and remaining 7 dogs were stage 4 CKD.

Estimation of renal biomarker

Table 1 presents a comparative evaluation of cystatin C concentrations in serum and urine between apparently healthy dogs (n=6) and those diagnosed with acute kidney injury (AKI) (n=6). Dogs with AKI exhibited significantly elevated serum creatinine (5.53 ± 0.96 mg/dL) and blood urea nitrogen (BUN) levels (104.58 ± 17.54 mg/dL) compared to healthy controls (0.56 ± 0.06 mg/dL and 12.25 ± 0.42 mg/dL, respectively), indicating marked azotaemia ($p < 0.01$). Dogs with AKI demonstrated significantly elevated serum cystatin C levels (2.10 ± 0.14 mg/L) compared to healthy controls (0.61 ± 0.25 mg/L). Urinary cystatin C concentrations were also markedly higher in the AKI group (0.641 ± 0.024 ng/mL) relative to controls (0.217 ± 0.035 ng/mL).

In the present study there was significant elevation in creatinine value in dogs with AKI compared to apparently healthy dogs. The reason for elevated creatinine in acute kidney injury (AKI) primarily reflects a sudden decline in glomerular filtration rate (GFR) due to abrupt damage to renal tubular structures [20, 31, 32].

As per the findings of the present study there was significant increase in the mean BUN value in dogs with AKI compared to apparently healthy dogs. The reason for elevated blood urea nitrogen (BUN) in dogs with acute kidney injury (AKI) primarily results from a reduced glomerular filtration rate (GFR), which impairs the kidney's ability to excrete

nitrogenous waste products derived from protein metabolism [5, 31, 32].

There was significant elevation in the mean value of serum cystatin C in dogs with AKI compared to apparently healthy dogs. Normal reference range of serum Cystatin C concentrations ranges between 0.57 to 1.2 mg/L in different age group of dogs having less than 1.6 mg/dL serum creatinine level [33]. The observed elevation in serum cystatin C levels may be attributed to its normal physiology being freely filtered by the glomerulus under healthy conditions. In the context of AKI, a decline in glomerular filtration rate (GFR) impairs cystatin C clearance, resulting in its accumulation within the bloodstream [1, 3, 20, 21, 24].

The mean urinary Cystatin C concentration in dogs diagnosed with acute kidney injury (AKI) was significantly elevated compared to that of apparently healthy dogs. The reason for elevation of urinary Cystatin C (uCysC) in dogs with acute kidney injury (AKI) is primarily due to proximal tubular damage, which disrupts the normal reabsorption and degradation of filtered Cystatin [10].

Table 1: Comparative analysis of renal biomarkers between Apparently healthy dogs and Dogs with AKI

Variables	Apparently healthy dogs (n=6)	Dogs with AKI (n=6)
Creatinine (mg/dL)	0.56±0.06	5.53±0.96**
Blood urea nitrogen (mg/dL)	12.25±0.42	104.58±17.54**
Serum cystatin C (mg/L)	0.61±0.25	2.1±0.14**
Urine cystatin C (ng/ml)	0.217±0.035	0.641±0.024**

Note: *-significant at P< 0.05 and **-significant at P< 0.01 compare to control group

Table 2 presents a stage-wise comparison of renal biomarkers between apparently healthy dogs (n=6) and those diagnosed with chronic kidney disease (CKD), stratified by IRIS stages. Progressive elevations in serum creatinine and blood urea nitrogen (BUN) were observed across CKD stages, with stage 4 dogs exhibiting the highest values (8.10 ± 0.70 mg/dL and 136.30 ± 26.18 mg/dL, respectively), consistent with advancing azotaemia and declining renal function. Serum cystatin C concentrations increased significantly from stage 1 (2.89 ± 0.84 mg/L) to stage 3 (4.26 ± 1.31 mg/L), before slightly declining in stage 4 (3.40 ± 1.01 mg/L). Urinary cystatin C levels were elevated in all CKD stages compared to healthy controls (0.217 ± 0.035 ng/mL), peaking in stage 3 (0.52 ± 0.016 ng/mL).

There was progressive elevation in mean creatinine value as stage advances in case of CKD compared to apparently healthy dogs. The reason for progressive elevation in creatinine value may be due to declining glomerular filtration rate (GFR) due to progressive nephron loss [13, 15, 17, 18, 23, 28].

Similarly, there was progressive elevation in mean BUN value in case of CKD compared to apparently healthy group. The reason for progressive elevation in BUN value may be due to declining glomerular filtration rate (GFR) due to progressive nephron loss. However, due to its sensitivity to extrarenal influences, BUN should be used in conjunction with more specific markers [13, 15, 18, 28, 30].

There was significant elevation in the mean value of serum cystatin C in dogs with CKD in all stages compared to apparently healthy dogs. Normal reference range of serum Cystatin C concentrations ranges between 0.57 to 1.2 mg/L in different age group of dogs having less than 1.6 mg/dL serum creatinine level [33].The reason for increase in serum Cystatin C concentrations in dogs with chronic kidney disease (CKD) is primarily attributed to a decline in glomerular filtration rate (GFR), which impairs the renal clearance of this low molecular weight protein. Cystatin C is produced at a constant rate by all nucleated cells and is freely filtered by the glomerulus under normal conditions. In CKD, compromised filtration leads to its accumulation in the bloodstream [1, 3, 20, 21, 24].

The mean urinary Cystatin C concentration in dogs diagnosed with CKD in all stages was significantly elevated compared to that of apparently healthy dogs. The primary reason for elevation of urinary Cystatin C (uCysC) in dogs with chronic kidney disease (CKD) may be due to proximal tubular damage, which disrupts the normal reabsorption and degradation of filtered Cystatin C [29].

Table 2: Comparative analysis of renal biomarkers between apparently healthy dogs and Dogs with CKD

Variables	Apparently healthy dogs (n=6)	Dogs with CKD			
		Stage 1 (n=6)	Stage 2 (n=6)	Stage 3 (n=6)	Stage 4 (n=7)
Creatinine (mg/dL)	0.56±0.06	1.35±0.022**	2.38±0.217**	3.72±0.21**	8.1±0.70**
Blood urea nitrogen (mg/dL)	12.25±0.42	30±1.31**	52.71±10.66**	74.27±6.02**	136.30±26.18*
Serum cystatin C (mg/L)	0.61±0.25	2.89±0.84**	3.81±0.95**	4.26±1.31**	3.4±1.01**
Urine cystatin C (ng/ml)	0.217±0.035	0.42±0.014**	0.51±0.025**	0.52±0.016**	0.46±0.018**

Note: *-significant at P< 0.05 and **-significant at P< 0.01 compare to control group

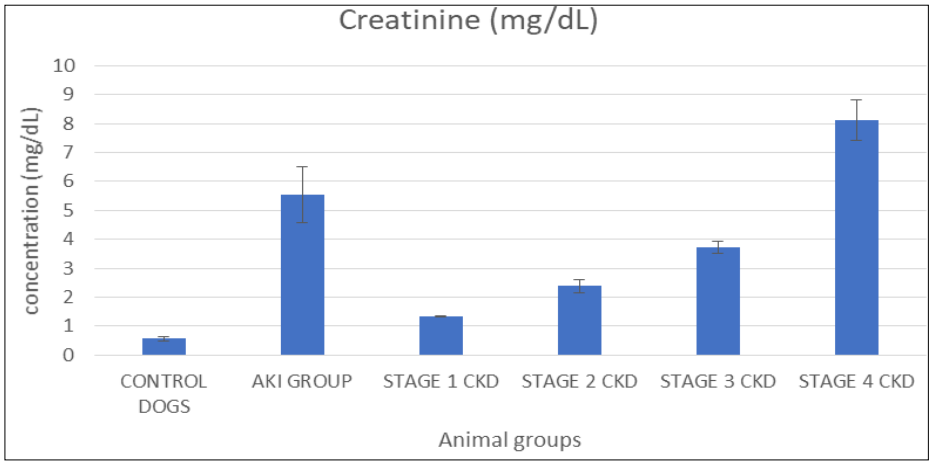

Fig 1: Creatinine (mg/dL) in dogs with Renal dysfunction

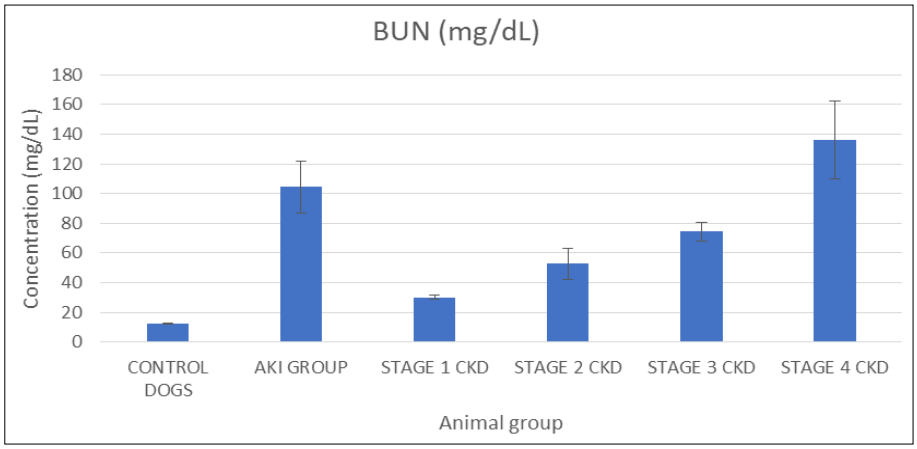

Fig 2: Blood Urea Nitrogen (mg/dL) in dogs with Renal dysfunction

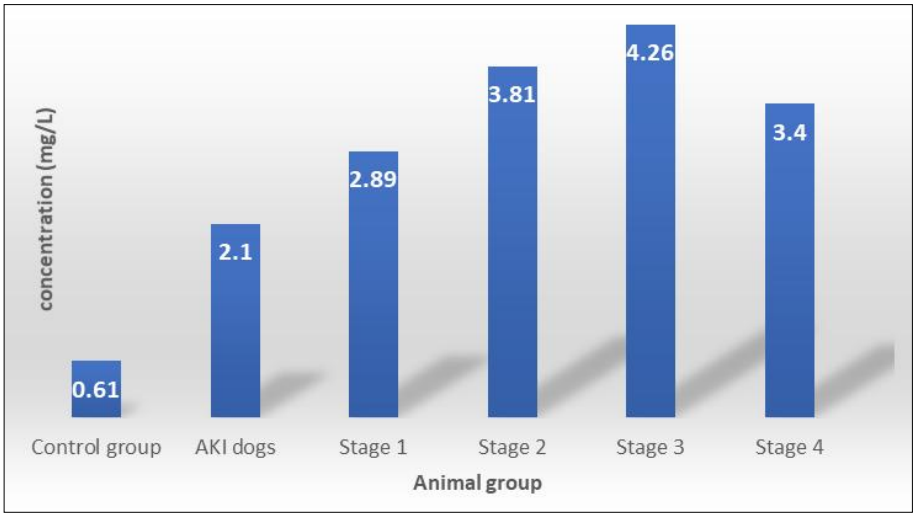

Fig 3: Serum Cystatin C in dogs with Renal dysfunction

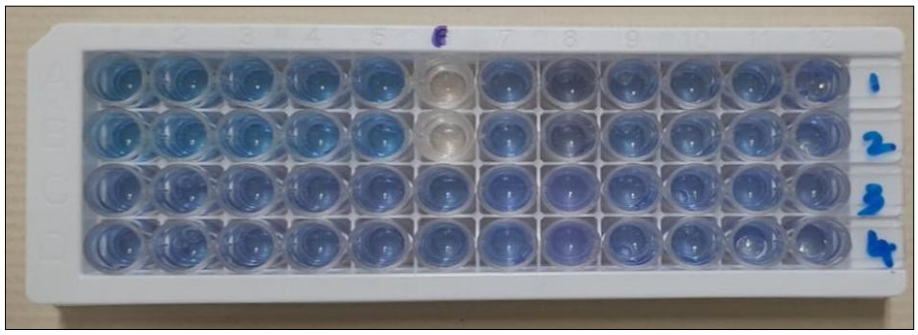

Plate 1: Cystatin C ELISA plate after adding Substrate solution A and Substrate solution B

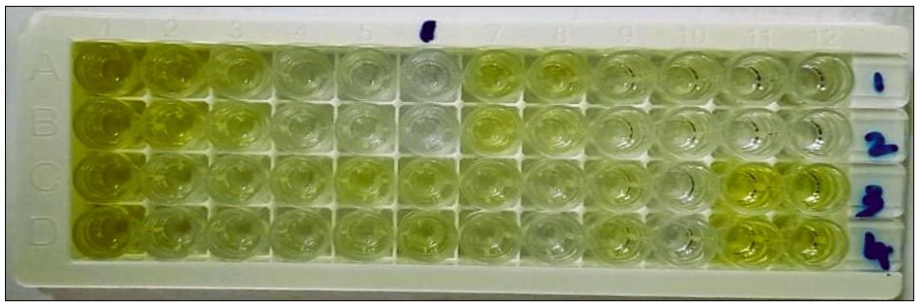

Plate 2: Cystatin C ELISA plate after adding Stop solution Calibration curve for serum Cystatin C ELISA

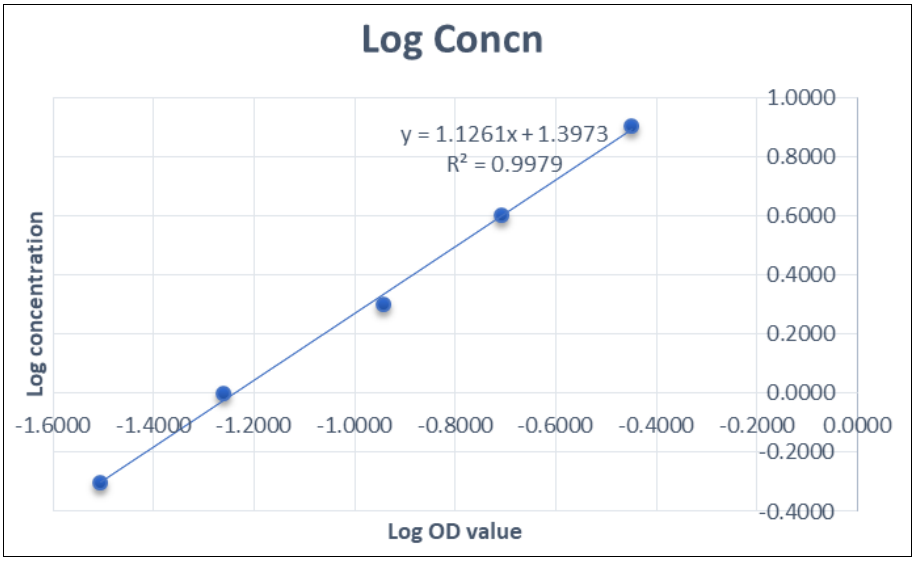

Fig 4: Urine Cystatin C in dogs with Renal dysfunction

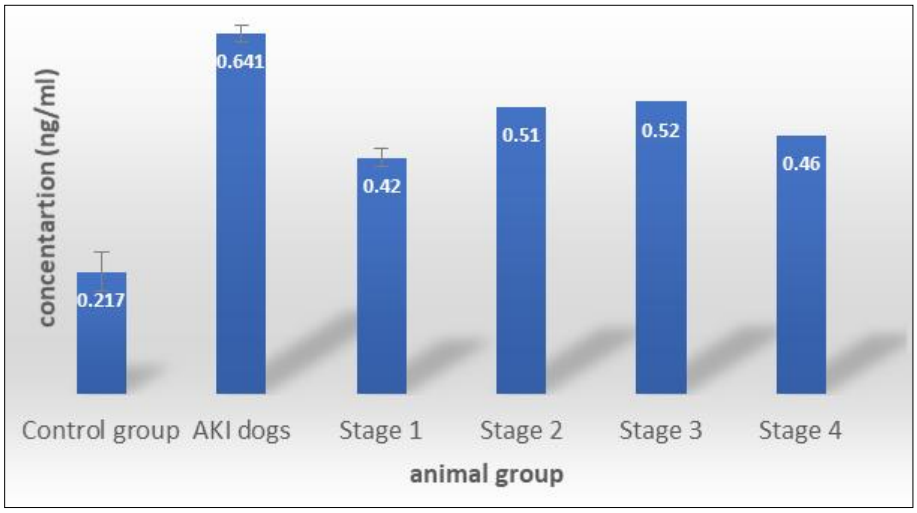

Fig 5: Cystatin C ELISA plate after adding Substrate solution A and Substrate solution B

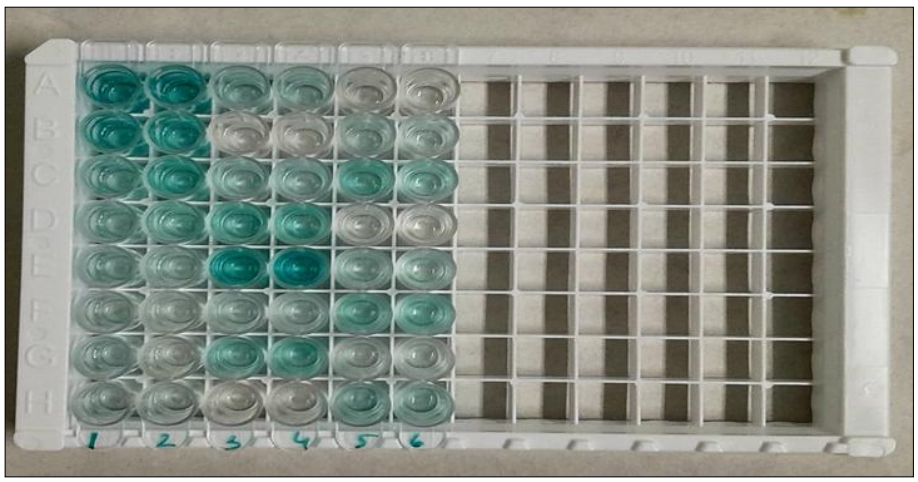

Plate 3: Cystatin C ELISA plate after adding Stop solution

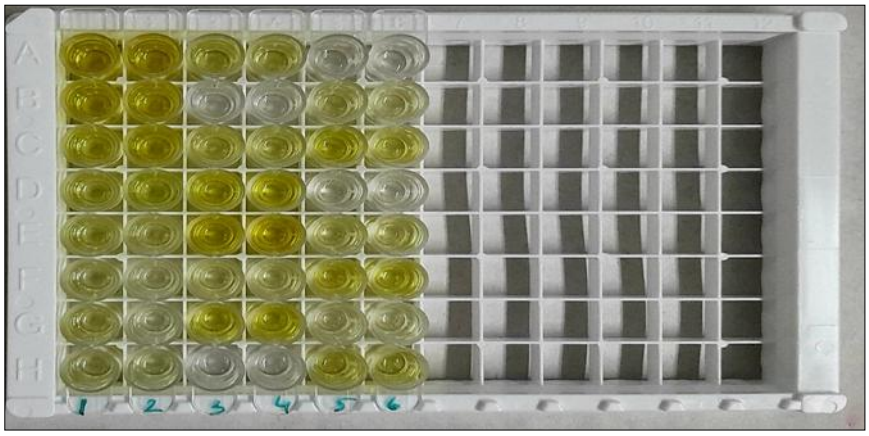

Plate 4: Calibration curve for Urine Cystatin C ELISA

Conclusion

Both serum and urine Cystatin C demonstrates significant promise as a sensitive and early biomarker for detecting renal dysfunction in dogs, particularly in cases where traditional markers such as serum creatinine may be confounded by extrarenal factors. Our findings underscore its utility in differentiating between stages of CKD and AKI, with consistent elevation correlating with disease severity and IRIS staging. Importantly, Cystatin C levels showed minimal influence from age, sex, or muscle mass, reinforcing its reliability across diverse canine populations. Integrating Cystatin C into routine diagnostic panels could enhance early detection, improve staging accuracy, and support timely therapeutic interventions. Further longitudinal studies are warranted to validate its prognostic value and explore its role in monitoring treatment response.

Conflict of Interest

Not available

Financial Support

Not available

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