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Evaluation of Serum Cystatin C and Associated Clinical and Biochemical Changes in Acute and Chronic Renal Dysfunction in Dogs

AbdelRahman M. Mahmoud[1]*, Sabry A. Mousa[2] and Hassan T. Elhamamsy[1]

[1] *Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.*

[2] *Department of Veterinary Clinical Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan..*

Abstract

KIDNEYS are vital for regulating hydration, erythropoiesis, elimination of toxins, and maintaining electrolyte balance. However, measuring certain parameters in dogs' serum or urine does not clearly distinguish between acute and chronic kidney dysfunction (AKD, CKD). This investigation aimed to approach the diagnostic and prognostic significance of serum cystatin C (Cys-C), a renal function marker, in dogs with AKD and CKD. The current study included 39 dogs total, 10 dogs served as the control group, and 22 dog were diagnosed with AKD and 7 dogs were diagnosed with CKD, with age range (1-13 years) and sex (22 males and 17 females). All dogs underwent clinical and physical examinations, a hematological profile including kidney and hepatic function tests, and urinalysis, as well as renal function marker (Cys-C). Hematology revealed anemia, thrombocytosis and leukocytosis in both AKD and CKD groups, while serum biochemistry revealed a significant increase in alkaline phosphatase (ALKP), cholesterol, glucose, ammonia, blood urea nitrogen (BUN), creatinine (Cr), potassium (K+), phosphorus levels, and Cys-C as well as significant decrease in BUN/Cr and Na^+/K^+ ratios in both groups, along with a decrease in calcium value and urine specific gravity in CKD. Urinalysis revealed a significant increase in urinary protein creatinine (UPC) ratio in both groups. Furthermore, Cys-C demonstrated sensitivity and greater specificity than creatinine as a prognostic indicator in AKD and CKD cases. As a result, we can conclude that the serum Cystatin C test holds a significant value as screening diagnostic and prognostic marker for renal dysfunction in dogs. Its results are correlated in different scenarios in dogs' cases, providing crucial information for diagnosing and prognosing various conditions from acute and chronic renal diseases.

Keywords: Cystatin C, Dogs, kidney dysfunction, Hemato-biochemical analysis.

Introduction

Acute kidney dysfunction (AKD) is a common and fatal condition, sudden onset of renal dysfunction which is reversible if treated properly, it's triggered by various clinical factors, including inflammation, infection, or toxins. Symptoms such as loss of appetite, vomiting, diarrhea, increased urination, and excessive thirst often occur simultaneously. [1].

Dogs of any age can have chronic kidney dysfunction (CKD), although older dogs are more likely to develop it. It is characterized by the presence of functional or structural changes in one or both kidneys for longer than three months [2]. It is a progressive condition in dogs, with a highly variable rate of development. The progression can be slow and steady or follow an episode of acute kidney injury (AKI) and often irreversible [3]. It is primarily an acquired condition with a variety of etiologies, such as infections (such as pyelonephritis and pyometra), glomerular diseases, nephrotoxicity, neoplasia, prior AKI, or urinary obstruction. Nevertheless, the etiology is unidentified at presentation and remains unclear throughout the disease course [4, 5].

Serum creatinine (Cr) is the primary marker for evaluating kidney function. However, Cr levels in the serum only increase after a 75% decline in the glomerular filtration rate (GFR). Furthermore, small-breed dogs have low muscle mass, resulting in normal serum Cr levels. Consequently, there is a need for more accurate and sensitive markers that are not influenced by non-renal factors for the early diagnosis and prognosis of renal dysfunction [6].

*Corresponding authors: AbdelRahman M. Mahmoud, E-mail: drabdelrahman716@gmail.com Tel.: 01146690949
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Serum Cystatin C (Cys-C) is a 13kD small protein that's produced by all body cells at fixed rate, serves as cysteine proteases inhibitor in dogs. It's superior GFR biomarker due to its low molecular weight and almost total resorption and degradation from proximal convoluted tubules, measurement of glomerular filtration rate is considered the gold standard for assessing renal function but techniques for the measurement of GFR are challenging because of the need for specialized equipment and the rigorous sampling procedures [7]. Serum Cys-C concentrations are significantly higher in dogs with renal failure than in clinically healthy dogs [8, 9]. Since serum Cys-C is not influenced by age, sex, diet, or muscle mass, it is a more accurate measure than serum Cr. Consequently, serum Cys-C in small-breed dogs may be a potential, sensitive renal marker [6, 10].

Evaluation the efficacy of serum Cystatin C as a biomarker for renal dysfunction in dogs is needed to address the necessity for more accurate and sensitive indicators that are not influenced by non-renal factors.

For this, the current study's objective is to evaluate the significance of serum Cys-C as a diagnostic and prognostic biomarker in dogs with AKD and CKD.

Material and Methods

Animals

Dogs suspected of renal disorders and brought to Private Small Animal Clinics and outpatient medical unit of Faculty of Veterinary Medicine; Cairo University were selected for this study after approval by the Local Ethical Committee as a part of PhD dissertation carrying the number of (VET CU16072020169). All owners consented and were informed to participate in the study.

A total of 39 dogs participated in this study, divided into three groups: (1) 10 healthy control dogs (age range: 2-8 years; 5 males and 5 females), (2) 22 dogs with AKD (age range: 1-12 years; 12 males and 10 females), and (3) 7 dogs with CKD (age range: 4-13 years; 5 males and 2 females). All dogs were fully vaccinated, dewormed, and fed a mixed diet.

AKD cases were categorized into four groups based on the underlying cause: obstruction, systemic inflammation (i.e. blood parasites), localized organ infection (i.e. pyelonephritis /pyometra), and miscellaneous (i.e. drug/diabetes/tumor). In contrast, CKD cases were classified into stages 3 and 4 according to international renal interest society (IRIS) guidelines to determine Pearson's correlation.

Samples

Paired blood and urine samples were collected from control and clinically affected dogs. All animals were examined for apparent clinical signs and vital signs (Respiratory rate (RR), Pulse rate (PR), Rectal temperature, Mucous membranes (M.M), and Lymph nodes (L.N)) were recorded, as shown in Tables 1 and 2.

Hemato-Biochemical assays

Whole blood was collected in EDTA, and lithium heparin tubes used in CBC (Procyte dx, IDEXX Laboratory automatic analyzer, inc., USA) and fasting ammonia (testing within maximum of 2 hours following sample withdrawal) using automatic analyzer (Catalyst one, IDEXX Laboratory, inc., USA).

Serum was obtained from plain tubes containing clotted blood by centrifugation at 3000 rpm for 10-15 minutes then stored at -20 ºC until analysis of selected biochemical parameters including liver and kidney functions (Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma-glutamyltransferase (GGT), Alkaline phosphatase (ALKP), Bilirubin (total), Blood urea nitrogen (BUN) & Creatinine), electrolytes (Sodium (Na^+), Potassium (K^+), Calcium (Ca^{++}) & Phosphorus), protein profile (Albumin (alb), Total protein (TP), Globulin (glob)), cholesterol, and glucose using automatic analyzers (Fujifilm Dri-chem NX500V, Japan) and (Catalyst, IDEXX Laboratory, inc., USA).

Serum Cystatin C (Cys-C) was evaluated quantitatively by enzyme linked immunosorbent assay (ELISA)-colorimetric using serum samples at CURP AGRIC lab, Egypt using (Canine Cys-c ELISA kit, Biotang, inc., USA).

Urine samples were collected by manual evacuation, catheterization or cystocentesis from both male and female dogs for urinalysis including dipstick chemistry (Medi-Test Combi 10 VET strips, Machery-Nagel, Duren, Germany) to detect Urine specific gravity (USG) and to detect urinary protein-to- creatinine ratio (UPC) was performed on fresh urine samples after centrifugation (using supernatant) as method of per [11].

Ultrasonography

Ultrasonographic examination was done using B-mode on transverse and longitudinal lanes on different U.S machines (Mindray DP 10 – China) and (Edan dus 60 & Vet – china) all of them equipped with micro-convex probes, frequencies ranging from 2-6.5 MHZ.

Statistical analysis

Data were analyzed by SPSS package version 20 (SPSS Inc., Chicago, IL, USA). Using independent samples t-test, expressed as mean values ± SE at levels of significance $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$.

Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), and other evaluation points of the serum Cystatin test as a screening diagnostic for kidney affection were assessed. Sonography was assigned as the gold standard test.

Kappa statistic (κ) was performed to measure the agreement between different screening tests based on the guidelines of [12] and adapted from [13]. The Landis & Koch scale was used to indicate the degree of agreement according to the Kappa value, with the scores divided into: < 0 no agreement; 0.0- 0.20 slight; 0.21- 0.40 fair; 0.41- 0.60 moderate; 0.61- 0.80 substantial; 0.81- 0.99 almost perfect; 1 perfect.

Pearson's correlation coefficient (*r*) test was used to evaluate the association between serum creatinine and cystatin c as variables. P values less than 0.05 were considered statistically significant.

Results

Most common clinical signs in AKD were anorexia, vomiting, behavioral changes, oliguria and anuria/ischuria, while patients with CKD showed anorexia, lethargy, weakness, weight loss, Abdominal distension and diverse neurologic and urologic manifestations (table1, Fig1-2).

Healthy control dogs exhibited normal vital signs, while the dogs with renal dysfunction showed alterations in their vital signs. These significant changes include variations in pulse rate, mucous membrane color, and lymph node characteristics as tabulated in table 2.

Abdominal ultrasound (US) examination results were delivered as mentioned in Fig. 3 & 4, as the most common findings in AKD were (hypertophic kidneys, engorged urinary bladder, stones, thickened cortical area, hydro-nephrosis and dilated pelvis) while in CKD (hypertrophic and/or hypotrophic hyper-echogenic kidneys, renal deformities, loss of cortico-medullary distinction and ascites).

Hematological parameters are tabulated in table 3. Hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC) levels are significantly decreased, whereas platelet and leukocyte counts are significantly higher in both the AKD and CKD groups compared to the control group. While packed cell volume (PCV) % is significantly higher in the AKD group.

Results of serum hepatic biochemical parameters are tabulated in table 4 to detect the possibility of hepatorenal syndrome (HRS) related cases. Significant elevation in ALKP, cholesterol, glucose and ammonia levels in both AKD and CKD compared to control dogs, while non-significant alteration was recorded in ALT, GGT, TP, albumin, and bilirubin.

BUN, creatinine, potassium, phosphorus, and UPC levels were significantly higher in both the AKD and CKD groups than in the control group. Similarly, the BUN/Cr and Na+/K+ ratios were significantly lower in both groups. Calcium levels and urine specific gravity are significantly lower in the CKD group than in the control group. Moreover, both groups exhibit a significant rise in serum Cys-C (Table 5).

In table (6), we are figuring out how effective serum Cystatin C is in diagnosing a certain condition compared to serum creatinine. Sensitivity means how good the test is at correctly identifying cases who have the condition. In this case, the sensitivity of the serum Cystatin C test is 63%, which means that it correctly identifies 63% of the dogs which actually have the condition. On the other hand, Specificity means how good the test is at correctly identifying cases who do not have the condition. The specificity of the serum Cystatin C test is 100%, which means that it rarely gives a wrong result for cases who do not have renal dysfunction.

Next, type 1 (false positive) and type 2 (false negative) errors. Serum Cystatin C test has a type 1 error rate of 0%, which is great because it means it hardly ever gives a false positive result. Type 2 error, or false negative, is when the test says you don't have the condition when you actually do. The serum Cystatin C test has a type 2 error rate of 37%, which means it misses identifying the condition in 37% of the cases.

Positive predictive value (PPV) indicate the likelihood that if the test is positive, the case actually has the condition. In this case, the PPV of the serum Cystatin C test is 100%, which is very good. While, negative predictive value (NPV) indicate the likelihood that if the test is negative, the case doesn't have the condition. The NPV of the serum Cystatin C test is 75%.

Overall, the accuracy of the serum Cystatin C test is 82.5%, which means it is pretty reliable. Also, the likelihood ratio negative, which is 0.368, but does not provide information on the likelihood ratio positive.

In table (7), the agreement between two different tests for measuring kidney function, serum Cystatin C, and serum creatinine, is being compared. The results show a Kappa value of 0.64, which indicates substantial agreement between the two tests. This means that there is a high level of agreement between the results obtained from the two tests.

Overall, comparing the results of two different tests for measuring kidney function and showing that there is a significant level of agreement between the two tests. This information is important to understand the reliability and accuracy of these tests in diagnosing kidney function.

In conclusion, the serum Cystatin C test is quite good as screening diagnostic and prognostic test for renal dysfunction cases, with high specificity, positive predictive value and significant substantial k agreement to creatinine. However, it does have a moderate false negative rate, meaning it may miss detecting the condition in some cases.

In table (8) and figure (5), the correlation between serum Cystatin C and serum creatinine tests' results in dogs' cases is shown. The table includes different scenarios of AKD and CKD.

The Pearson's Correlation (r) value tells us how closely related the two tests are. A positive value means they are positively correlated, while a negative value means they are negatively correlated. The closer the value is to 1 or -1, the stronger the correlation.

For example, in the case of AKD related to systemic infections, the r value is 0.59, which means there is a moderate positive correlation between serum Cystatin C and serum creatinine tests' results. This could indicate that in cases of systemic infection, both tests may show similar results.

On the other hand, in the case of localized infection (Pyelonephritis/Pyometra), the r value is -0.79, indicating a strong negative correlation. This means that in cases of infection, the results of the two tests are likely to be different from each other.

P value shows if the correlation is statistically significant. P value less than 0.05 is considered significant, meaning that the correlation is unlikely to have occurred by chance. For example, in the case of localized infections (Pyelonephritis/Pyometra), the P-value is 0.062, which is close to 0.05. This suggests that the negative correlation between the two tests may be significant in cases of infection. Overall, this table tells how serum Cystatin C and serum creatinine tests' results are correlated in different scenarios in dogs' cases, providing valuable information for diagnosing and treating various conditions.

Discussion

This research underscores the valuable role of cystatin C as a dependable biomarker for assessing kidney function, providing benefits over conventional creatinine-based methods.

In this research, the frequently noted clinical symptoms of AKD and CKD were general, including lethargy, decreased appetite, vomiting, diarrhea, oligo/polyuria or anuria/ischuria, and polydipsia, consistent with previous studies [1, 14, 15]. These symptoms arise from uremic toxins, organ involvement, and complications, indicating disease severity. [16, 17, 18, 19].

Normal heart rate vary among dogs based on breed, age, and size, typically falls between 60 and 160 beats per minute (bpm). However, renal impairment can affect heart rate due to changes in blood volume, fluid retention, electrolyte imbalances, stress or compensatory reactions to lower cardiac output [20, 21, 22].

Presence of variable US findings were observed in our study including renal and urinary bladder stones, hyperthrophic or hypotrophic kidneys, loss of cortico-medullary distinction, ascites and deformities, which deliver a relation between functional and structural renal status, that's why abdominal ultrasounds scans are recommended for dogs with renal disease or azotemia [23].

Mucous membrane (color) was observed as pale and congested which is in harmony with [24, 25] who observed clinical sign like pallor in dogs with CKD. Dogs with renal disease exhibited clinical signs such as pale mucous membranes [26]. A possible reason for congested mucous membrane of dogs suffered with renal failure might be due to uremia and fever [27].

Consistent with prior studies in dogs [1, 14, 28], anemia at presentation was identified as a negative prognostic factor. Although anemia is typically associated with CKD, dogs with AKD can develop anemia due to gastrointestinal bleeding, inflammation, and reduced erythropoietin production. The AKD group exhibited an increased PCV% compared to the control group, indicating dehydration or hemo-concentration. Dehydration is common in CKD, often due to the patients' inability to drink sufficient water to offset increased urinary losses [29]. Numerous studies have examined platelet parameters as indicators of AKD and its consequences. A higher mean platelet count suggests increased platelet turnover and activity, which may indicate more severe inflammation and pose a risk for total vascular death [30].

In relation to WBCs count, leukocytosis has been linked to an increased risk of acute kidney disease (AKD) and may be connected with neutrophilia and a pro-inflammatory activity as a component of the inflammatory immune response [31]. A substantial correlation was shown between an elevated neutrophil–lymphocyte ratio (NLR) and a higher risk of AKD and death [32, 33].

Both groups exhibited significantly elevated levels of random blood glucose, cholesterol, and ALKP activities. These findings may suggest complications such as pancreatitis, liver damage, or stress. Alternatively; these elevated levels may indicate the severity of the disease, as evidenced by extra-renal consequences [1, 3, 34, 35]. The bacterial breakdown of urea to ammonia may explain the elevated ammonia levels observed in renal failure [36], even though the levels in the AKD group remained within normal limits.

Elevated BUN and creatinine levels indicate impaired kidney function, as the kidneys typically filter and excrete urea and creatinine. This finding is consistent with the earlier report by [37]. In line with previous studies [1, 14], serum creatinine concentration at presentation was not associated with mortality; thus, prognosis should not be based on serum creatinine levels at presentation. A significant decrease in the BUN/Cr ratio and the Na^+/K^+ ratio can provide insights into the underlying cause of kidney dysfunction. A lower ratio may suggest intrinsic kidney disease and is an important indicator of electrolyte balance and renal function [38].

Both groups had phosphate and potassium levels that were noticeably higher. However, because of impaired renal function, the CKD group has significantly lower calcium levels than the control group [2, 29, 37, 39].

In comparison to the control group, UPC was considerably significant in AKD and CKD groups. Severe proteinuria, a typical indicator of renal failure that can occur in both acute and chronic situations. [40]. Excessive protein excretion in the urine is a common sign of chronic kidney disease (CKD) and persistent albuminuria, as well as chronic nephropathies [41, 42]. When compared to dogs with a UPC ratio of less than one, dogs with a UPC greater or equal to one were more likely to experience a uremic crisis and eventually death [43]. Decreased urine specific gravity in the CKD group suggests compromised renal concentrating ability, subjected to either polyuria or rehydration procedures and in animals whose kidneys have lost the ability to concentrate urine [38, 39, 44].

Cystatin C (Cys-C) levels in both the AKD and CKD groups are significantly higher compared to control group. It was reported that the cystatin C to creatinine ratio can differentiate dogs with renal disease from healthy dogs [7, 10, 45]. The present results suggest that cystatin C can be used as early diagnostic marker in dogs with CKD as it's highly elevated in comparison to serum creatinine. This finding aligns with [46], who noted that the sensitivity and specificity of Cys-C were significantly higher than those of serum creatinine. In our study, serum cystatin C demonstrates higher specificity in detecting CKD.

In AKD associated with localized infections (pyelonephritis/pyometra), cystatin C and creatinine was negatively correlated, highly elevated creatinine level was significantly associated with prolonged hospitalization and/or death in pyometra. Therefore, this parameter was an excellent marker of morbidity and mortality in dogs suffering from pyometra as reported by [47].

In stage 3 of CKD the correlation between cystatin C and creatinine was negatively correlated, this means that serum cystatin C is dramatically increased in CKD and considers an early indicator of loss of kidney functions, and it is better than serum creatinine in detecting stage 3 CKD as documented by [48].

Conclusion

The data unequivocally demonstrate significant disparities in renal function tests between the control group and the groups with kidney dysfunction (AKD and CKD). The elevated levels of BUN, creatinine, potassium, phosphate, cystatin C, and the urine protein-to-creatinine ratio in both AKD and CKD groups are indicative of impaired renal function. Notably, serum cystatin C levels are significantly elevated in both AKD and CKD groups, underscoring its potential as a biomarker for diagnosing kidney disease in clinical settings. Moreover, cystatin C has been proven to be more specific than serum creatinine for the early diagnosis of CKD and for assessing the severity of AKD in dogs.

Recommendations for future studies:

Our findings highlight a number of knowledge gaps about participation in research that might benefit from additional study, including realist evaluation to strengthen and validate the theory we have involved here:

1. A larger sample size that increases the statistical power and generalizability of the findings.
2. Follow up of alive dog cases with AKD and CKD over time to assess the predictive value of cystatin C for disease progression and outcomes.
3. Comparison with other biomarkers: Compare cystatin C to other established biomarkers of renal function to determine its relative sensitivity and specificity.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethical committee guidelines of the Faculty of Veterinary Medicine, Cairo University, Egypt (ethics approval number; VET CU16072020169).

TABLE 1. Clinical symptoms of dogs suffering from AKD and CKD.

Clinical symptoms	AKD		CKD	
General Signs				
Anorexia, Lethargy, and weakness	18/22	81.8%	7/7	100%
Weight loss	3/22	13.6%	4/7	57.1%
Abdominal distension	4/22	18.2%	3/7	42.8%
Gastrointestinal signs				
Vomiting	6/22	27.3%	1/7	14.3%
Diarrhea	4/22	18.2%	2/7	28.5%
Melena	3/22	13.6%	1/7	14.3%
Hematemesis	1/22	4.5%	1/7	14.3%
Hematochezia	0/22	0%	1/7	14.3%
Neurological Signs				
Behavioral or Mental changes	15/22	68.2%	4/7	57.1%
Disorientation, stupor or coma	1/22	4.5%	2/7	28.5%
Generalized seizures	2/22	9.1%	1/7	14.3%
Urinary signs				
Polyuria/Polydipsia	7/22	31.8%	3/7	42.8%
Oligouria/Anuria/Ischuria	12/22	54.5%	3/7	42.8%
Hematuria	3/22	13.6%	1/7	14.3%

TABLE 2. Vital signs in healthy control dogs compared to kidney dysfunction dogs

Groups / Vital signs	Control (n=10)	Kidney dysfunction (n= 29)	
		AKD (n=22)	CKD (n=7)
	Mean ± SE	Mean ± SE	Mean ± SE
RR (resp./min)	29.7±2.91	33±1.93	31.29±3.48
Pulse (beat/min)	85.7±8.94	121±4.01 ***	114.57±8.65**
Temp (C^o)	38.74±0.42	38.64±0.23	37.86±0.48
M.M	Rosy red	Congested to pale	Pale to rosy red
L.N	Symmetric, soft, non-enlarged	Normal to enlarged	Normal

Means within a row bearing different superscripts show different levels of significance: $p \leq 0.001$ (***), $p \leq 0.01$(**) and $p \leq 0.05$ (*)

TABLE 3. Hemogram in healthy control dogs compared to kidney dysfunction dogs

Groups / Parameters	Control (10)	Kidney dysfunction (n= 29)	
		AKD (n=22)	CKD (n=7)
	Mean ± SE	Mean ± SE	Mean ± SE
RBCs (10^6/µl)	5.91±1.19	6.18±0.28	4.99±0.25
HB (g/dl)	11.42±0.74	9.72±0.47 ***	8.82±0.59***
Platelets (10^3/µl)	246±83.80	371.68±24.41**	262.57±33.0
PCV %	36.6±0.69	42.8±1.53 *	38.84±3.93
MCV (fl)	64.66±2.08	72.8±4.37	78.42±7.61
MCH (Pg)	20.07±0.42	16.44±1.06	17.93±1.32
MCHC (g/dl)	31.83±0.40	23.06±1.11***	24.28±2.97 *
RDW %	13.91±0.99	13.8±0.62	15.14±1.64
WBCs (10^3/µl)	10.55±0.39	15.67±1.96 *	15.84±2.93
NØ (10^3/µl)	6.09±0.32	7.57±0.97 ***	7.46±1.62***
EØ (10^3/µl)	0.32±0.19	0.67±0.06 ***	0.74±0.15 *
LØ (10^3/µl)	3.29±0.14	6.11±0.97 **	6.24±1.82
MØ (10^3/µl)	0.85±0.50	1.29±0.22	1.4±0.27

Means within a row bearing different superscripts show different levels of significance: $p \leq 0.001$ (***), $p \leq 0.01$(**) and $p \leq 0.05$ (*)

TABLE 4. Hepatic biochemical tests in healthy control dogs compared to kidney dysfunction dogs

Groups / Hepatic enzymology and function tests	Control (10)	Kidney dysfunction (n= 29)	
		AKD (n=22)	CKD (n=7)
	Mean ± SE	Mean ± SE	Mean ± SE
ALT (u/l)	46.5±9.95	44.55±4.43	51.29±5.89
AST (u/l)	34.4±5.08	42.41±3.29	48.71±7.77
ALP (u/l)	75.7±17.39	112.82±12.60	191.71±22.65 ***
GGT(u/l)	7.13±0.91	8.23±0.53	7.71±0.97
Bilirubin (mg/dl)	0.26±0.04	0.28±0.02	0.29±0.06
TP (g/dl)	6.52±0.26	6.5±0.31	7.11±0.49
Albumin (g/dl)	3.37±0.15	3.22±0.14	3.37±0.18
Globulin (g/dl)	3.15±0.28	3.1±0.26	3.74±0.53
A/G	1.21±0.2	1.31±0.16	1.04±0.18
Cholesterol (mg/dl)	152.8±13.77	202.14±13.23 *	222.71±26.71 *
Glucose (mg/dl)	95.5±4.88	156.95±12.15 ***	140.57±9.11 ***
Ammonia (mcg/dl)	17.3±1.84	38.41±2.51 ***	63.14±13.42 *

Means within a row bearing different superscripts show different levels of significance: $p \leq 0.001$ (***), $p \leq 0.01$(**) and $p \leq 0.05$ (*)

TABLE 5. Kidney functions in healthy control dogs compared to kidney dysfunction dogs

Groups / Kidney Functions	Control (10)	Kidney dysfunction (n= 29)	
		AKD(n=22)	CKD (n=7)
	Mean ± SE	Mean ± SE	Mean ± SE
Renal function biomarker			
Cystatin C (mg/l)	0.59±0.21	1.35±0.11 ***	2.84±0.10 ***
Renal enzymes			
BUN (mg/dl)	20.34±2.23	109.09±5.52 ***	101.0±7.36 ***
Creatinine (mg/dl)	1.12±0.07	8.68±0.50 ***	6.74±1.17 **
BUN/Creat. ratio	18.31±1.79	13.28±0.94 **	19.57±5.02 ***
Electrolytes			
K^+ (mmol/l)	4.51±0.67	6.25±0.18 ***	6.35±0.20 ***
Na^+ (mmol/l)	143±4.83	147.36±2.86	144.86±5.70
Na^+/K^+ ratio	32.33±4.97	24.13±1.04 ***	23.03±1.43 ***
Phosphorus (mg/dl)	4.42±1.06	7.39±0.58 ***	10.16±1.45 **
Calcium (mg/dl)	9.9±0.50	10.62±0.31	8.64±0.33**
Urinalysis			
UPC ratio	0.23±0.14	0.84±0.08 ***	1.09±0.15 ***
Urine specific gravity	1.024±0.0021	1.024±0.0018	1.009±0.004***

Means within a row bearing different superscripts show different levels of significance: $p \leq 0.001$ (***), $p \leq 0.01$(**) and $p \leq 0.05$ (*)

TABLE 6. Evaluation of the diagnostic values of serum Cystatin C compared to serum creatinine tests' results.

	Serum Cystatin
Sensitivity	63%
Specificity	100%
Type 1 error (false positive)	0
Type 2 error (false negative)	37%
Apparent prevalence	30%
PPV	100%
NPV	75%
Accuracy	82.5%
Likelihood ratio positive	-
Likelihood ratio negative	0.368

PPV: positive predictive value; NPV: negative predictive value; True prevalence determined by serum creatinine level was 47.5% in dogs.

TABLE 7. The agreement between serum Cystatin C and serum creatinine tests' results.

Diagnostic Test	Kappa value (*κ*)	Agreement	*P*-value
serum Cystatin *vs* serum Creatinine	0.64	Substantial	<0.0001

Scores divided into: < 0 no agreement; 0.0- 0.20 slight; 0.21- 0.40 fair; 0.41- 0.60 moderate; 0.61- 0.80 substantial; 0.81- 0.99 almost perfect; 1 perfect.

TABLE 8. Correlation between serum Cystatin C and serum creatinine tests' results.

Dogs' cases	Pearson's Correlation (*r*)	*P*-value
AKD		
Obstruction	0.32	0.489
Systemic infection (blood parasites)	0.59	0.286
Localized infection (Pyelonephritis/Pyometra)	-0.79	0.062
Miscellaneous (drug/diabetes/tumor)	-0.45	0.552
CKD		
Stage 3	-0.97	0.154
Stage 4	0.20	0.802

r values; 1= perfectly positive, -1= perfectly negative that can be linear when plotted on scatterplot
P values less than 0.05 were considered statistically significant

Fig.1. A) 7 year-old, female Dalmatian dog suffered from hyperglycemia, melena and depression as acute pancreatitis associated with AKD. **B)** 12 year-old, female German shepherd suffered from external parasitism, poor BCS, and nervous manifestations (seizures) associated with AKD**. C)** 7 year-old, male Pitbull showed crouching, difficult urination and urine retention due to penile bite wound that led to AKD**. D)** 3 year-old, male Pitbull noted for distended belly and ischuria by examination para-phimosis was detected and thereby resulted in AKD. **E)** Palor of mucous membrane in 5 year-old, German shepherd female dog due to severe *anaplasma* spp. Infection (snap 4dx test) and huge mammary tumor showing signs of AKD [resolved by blood transusion and surgerated for mammary tumor]. **F)** 5 year-old, male German shepherd suffered from a post-surgical infected castration wound which led to complete urine retention and AKD [resolved by catheterization]. **G)** 1 year-old, male T. Mastiff suffered from demodecosis which had been treated using NSAID (Diclofenac as per owner) toxicity which resulted in AKD

Fig.2. A) 13 year-old, male Beagle suffered from hemorrhagic gastroenteritis secondary to kidney tumor associated with CKD**. B)** 4 year-old, female Golden Retriever suffered from pulmonary edema, *Ehrilichia* spp. detected using blood film within a neutrophil, presence of isothenuria and Proteinuria according to urine dip stick all out of CKD. **C)** 6 year-old, female Rottweiler suffered from ascites secondary to CKD**.**

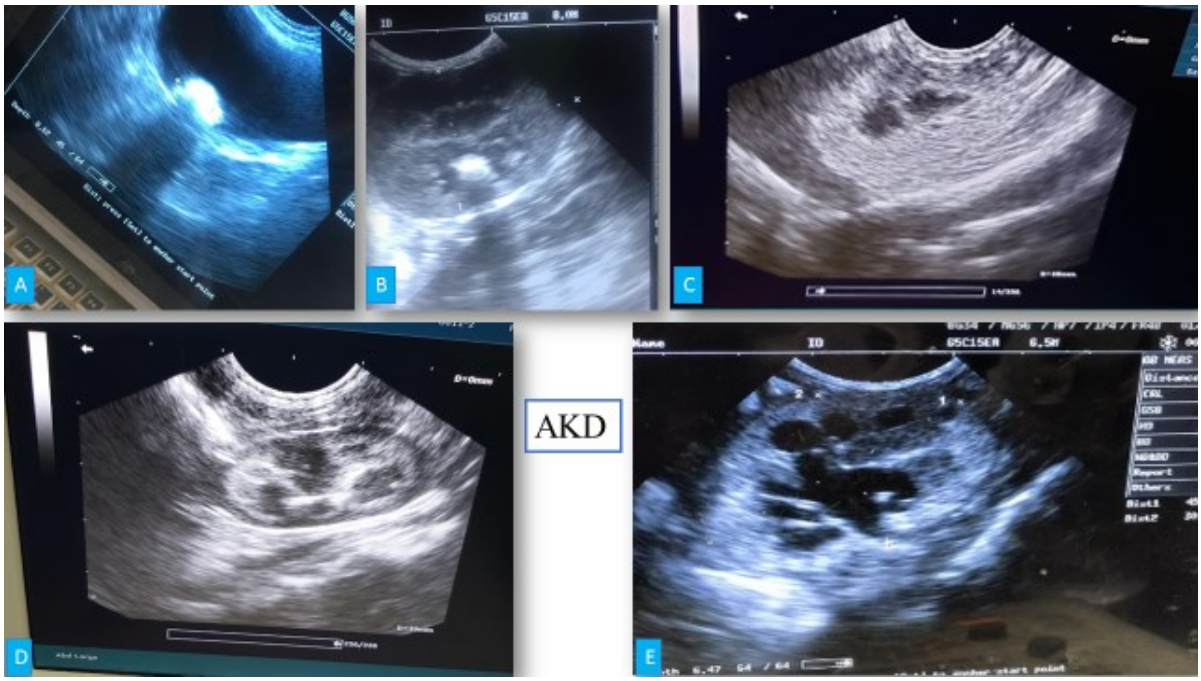

Fig.3. Ultrasonographic findings in cases related to AKD as follow **A) & B)** Showing presence of hyperechoic calculi present at the level of urinary bladder and renal pelvis measuring 17x9 mm and 11x6mm respectively. **C)** Large sized, hyper-echogenic right kidney with cortical thickening in conjugate with pyometra [AKD]. **D)** Sagittal sonogram of left kidney showing a loss of cortico-medullary differentiation with heterogenic echogenicity of renal cortex. **E)** Moderate hydronephrosis and low corticomedullary junction differentiation as a result of incomplete urine retention in a dalmation male dog.

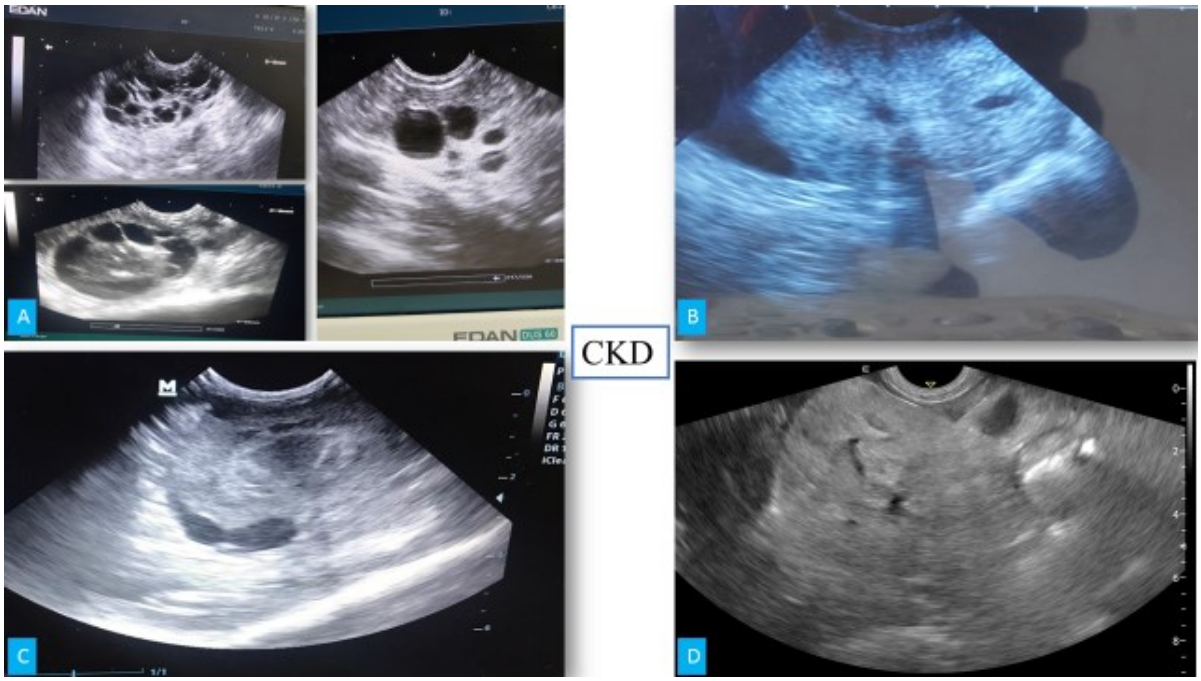

Fig.4. A) Different Sonographic scans revealed polycystic, hydro-nephrotic kidneys in a 12 year-old cocker-spaniel dog with CKD. **B)** Irregular, hyperechoic small sized left kidney with peri-renal ascetic fluid in a 6 year-old, female Rottweiler. **C)** Right kidney showing irregular borders and nephrotic rim in conjugate with peri-renal ascetic fluid in a 7 year-old, male griffon dog with CKD. **D)** Sonographic exam showing distorted right kidney with increasing size with no cortico-medullary distinction which submitted later for aspiration and cytology indicated pleomorphic nucleated cells suggesting renal tumor in a 13 year-old, male Beagle dog.

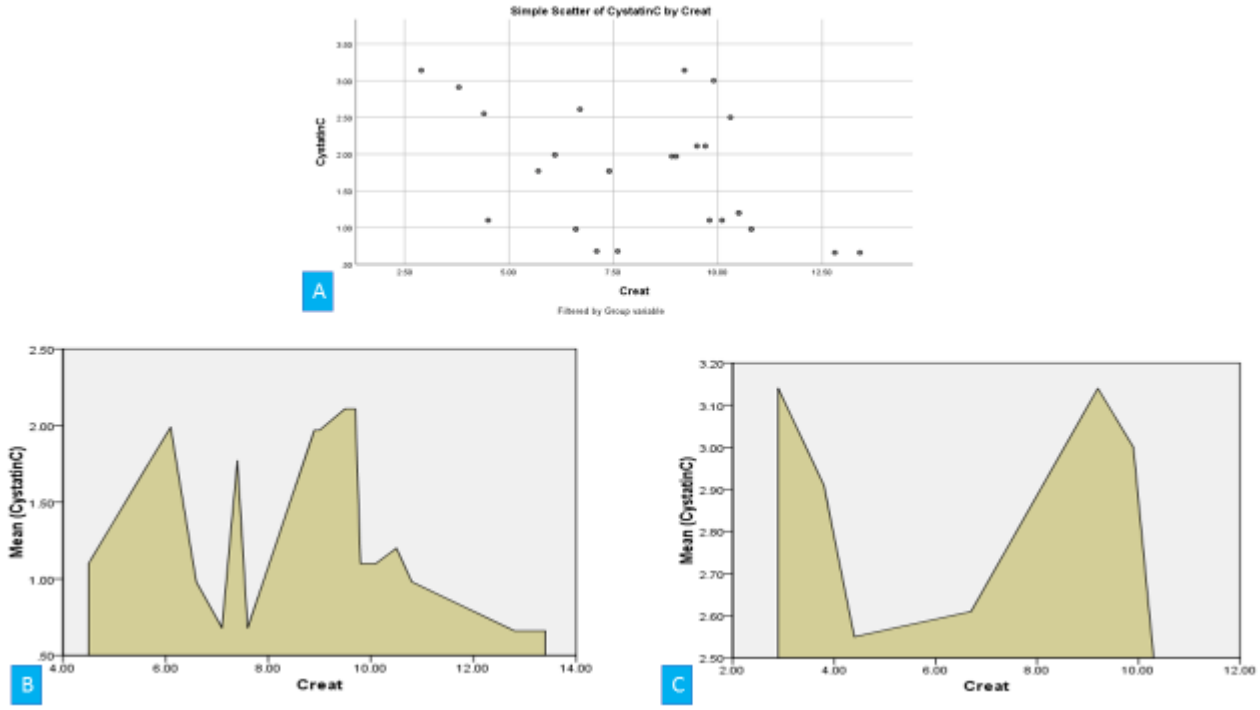

Fig.5. A) According to the scatter graph it revealed a negative correlation between cystatin C and sCr in most cases of renal insufficiency. **B)** The AKD area mapping graph suggests that cystatin C can be used as a possible prognostic marker for acute renal insufficiency. As shown, creatinine is increasing in all cases of AKD regardless of the cause, while cystatin C indicated normal values in the same calculated cases (AKD caused by stated obstructive or pyometra related cases). **C)** On the other hand CKD group showed increase in serum Cystatin C with no decline beyond the normal reference range which indicate that cystatin c is confirmatory marker for a damaged renal tissue along with creatinine.

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تقييم للسيستاتين سى فى السيرم مع التغيرات السريرية والكيميائية الحيوية في اختلال وظائف الكلى الحاد والمزمن في الكلاب

عبدالرحمن مصطفي محمود[1]، صبري احمد موسي[2] و حسن طه الحمامصي[1]

[1] قسم الامراض الباطنة والمعدية، كلية الطب البيطرى، جامعة القاهرة، مصر.
[2] قسم العلوم الطبية السريرية البيطرية، كلية الطب البيطري، جامعة العلوم والتكنولوجيا الأردنية، إربد 22110، الأردن

الملخص

تلعب الكلى دورًا حاسمًا في إدارة الماء، وإفراز الهرمونات الضرورية لإنتاج خلايا الدم الحمراء، والتخلص من السموم، والحفاظ على التوازن المناسب للكهارل. لا يمكن لقياسات بعض المعلمات في مصل الكلاب و/أو بولها أن تفرق بشكل كامل بين خلل الكلى الحاد والمزمن (AKD، CKD). نحن نهدف إلى دراسة القيمة التشخيصية والتنبؤية للسيستاتين سى فى السيرم ؛ مؤشر وظيفة الكلى في الكلاب مع AKD وCKD. شملت دراستنا 39 كلباً، حيث كانت 10 كلاب بمثابة مجموعة مراقبة، و22 كلباً عانت من مرض الكلى المزمن، و7 كلاب عانت من مرض الكلى المزمن بمتوسط عمر (1 - 13 سنة) والجنس (22 ذكراً و17 أنثى). يتم إجراء فحص سريري وجسدي لجميع الكلاب، وفحص أمراض الدم واختبارات وظائف الكلى والكبد، وتحليل البول بالإضافة إلى علامة وظائف الكلى (السيستين سي). كشفت أمراض الدم عن فقر الدم، كثرة الصفيحات مع زيادة عدد الكريات البيضاء في كل من مجموعتي AKD وCKD، في حين كشفت الكيمياء الحيوية في الدم عن زيادات كبيرة في ALP والكوليسترول والجلوكوز والأمونيا وBUN والكرياتينين والبوتاسيوم ومستويات الفوسفور وعلامة وظائف الكلى (السيستاتين سى) مع انخفاض كبير في BUN/Cr. ونسب Na+/K+ في كل من مجموعتي AKD وCKD إلى جانب انخفاض قيمة الكالسيوم والجاذبية النوعية للبول في مرض الكلى المزمن. كشف تحليل البول عن زيادة كبيرة في نسبة الكرياتينين البروتيني البولي (UPC) في كلا المجموعتين. أيضًا، أثبت السيستاتين سى أنه أكثر حساسية ونوعية من الكرياتينين كمؤشر تنبؤي في حالات AKD وCKD.

لذلك يمكننا أن نستنتج أن استخدام السيستاتين سى يمكن أن يكون علامة تشخيصية وتنبؤية مفيدة في التمييز بين التمييز بين بعض الحالات المرتبطة الخلل الكلوي الحاد والمزمن في الكلاب.

الكلمات المفتاحية: السيستاتين سي، الكلاب، خلل وظائف الكلى، التحليل الكيميائي الحيوي.