REPUBLIC OF TÜRKİYE
ERCIYES UNIVERSITY
INSTITUTE OF HEALTH SCIENCE
DEPARTMENT OF VETERINARY INTERNAL MEDICINE

DETERMINATION OF SERUM AND URINARY CLUSTERIN LEVELS IN CATS WITH RENAL FAILURE

Prepared By
Alfatih Mohammed Ahmed ABOZAID

Supervisor
Prof. Dr. Vehbi GÜNEŞ

Master's Thesis

January 2023
KAYSERİ

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January 2023
KAYSERİ

COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in this study was obtained in accordance with academic and ethical rules. I also state that I have fully cited and referenced all materials and results that are not inherent in this study, as these rules and behaviour require.

Alfatih Mohammed Ahmed ABOZAID

Signature ……

SUITABILITY FOR GUIDE

The MSc thesis entitled "**DETERMINATION OF SERUM AND URINARY CLUSTERIN LEVELS IN CATS WITH RENAL FAILURE** "has been prepared in accordance with Erciyes University Health Sciences Institute thesis preparation and writing guide.

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BÖBREK YETMEZLİĞİ OLAN KEDİLERDE SERUM ve İDRAR CLUSTERİN DÜZEYLERİNİN BELİRLENMESİ

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ÖZET

Bu tez çalışmasının amacı, üriner sistem problemi olan ve özellikle akut ya da kronik seyirli böbrek hastalığı olan kedilerde Clusterin seviyelerini belirlemek ve ilgili parametrenin böbrek hastalığına duyarlı bir biyobelirteç olup olmadığını araştırmak için yapılmıştır.

Kedilerin kronik böbrek yetmezliği (KBY), akut böbrek yetmezliği (ABY), alt üriner sistem hastalıklarında Clusterin'in kan ve idrar düzeylerinin etkilenebileceği düşünülmüştür. Clusterin parametresinin farklı olgularda düzeylerindeki varyasyonların yanı sıra, klinik, biyokimyasal, üriner, ultrasonografik ve bazı kan parametleri ile olan korelasyonları da incelenmiştir.

Bu amaçla çalışma ve kontrol grubunu içeren kediler araştırmanın hayvan materyalini oluşturdu. Çalışma grubunda, Erciyes Üniversitesi Eğitim ve Araştırma Hastanesine getirilen çeşitli ırklardan kronik böbrek yetmezliği (n=10), akut böbrek yetmezliği (n=7), alt üriner sistem hastalığı (n=8), akut böbrek yetmezliği ile birlikte alt üriner sistem hastalığı (n=12), kronik böbrek yetmezliği ile birlikte alt üriner sistem hastalığı (n=9), olan toplam 46 kediden kan örnekleri alındı. Ayrıca farklı 23 adet kediden idrar örnekleri toplandı. Kontrol grubu örnekleri ise aynı merkeze genel sağlık kontrolü amacıyla getirilen 10 adet sağlıklı kediden alındı. Hasta ve kontrol gruplarından alınan serum ve idrar örnekleri yarışmalı ELISA yöntemi ile analiz edildi. Ortalama serum Clusterin düzeyinin tüm hasta kedilerde 42.87 µg/ml (34.51-57.17), sağlıklı kedilerde 36.42 µg/ml (29.08-48.39) olduğu belirlendi. Hasta grubunun ortalama serum Clusterin düzeyi istatiksel olarak ($P<0.05$) sağlıklı grubun ortalamasından daha yüksekti. Hasta kedilerin idrardaki ortalama Clusterin miktarları 20.59 µg/ml (16.83-25.91) idi. Bu düzey hasta serumu ve sağlıklı kedi serumunda ölçülen Clusterin düzeylerinden istatistiksel olarak önemli ölçüde daha düşük bulundu ($p<0.001$).

Kronik böbrek yetmezliği olan kediler (ortalama Clusterin miktarları 84.79 ± 61.87µg/ml), akut böbrek yetmezliği olan kedilere (38.80 ± 15.18 µg/ml) göre istatiksel olarak anlamlı derecede daha yüksek Clusterin seviyelerine sahipti ($p<0.001$). Çalışmaya dahil edilen LUTD'li kedilerde ortalama serum Clusterin konsantrasyonu (23.24 ± 69.12 µg/ml), ARF+LUTD olan kedilerde ortalama serum clusterin konsantrasyonu (37.13 ± 10.94 µg/ml), CKD+LUTD olan kedilerde ise ortalama serum Clusterin konsantrasyonu (54.75 ± 22.61 µg/ml) olduğu belirlendi

Tüm hastalarda serum Clusterin ve BUN düzeyleri istatiksel anlamda pozitif bir korelasyon sergilerken ($P<0.001$, $r=0.756$), Clusterin ve kreatinin düzeyleri ise istatiksel anlamda çok daha güçlü ve pozitif bir korelasyon gösterdi. ($P<0.001$, $r=0.829$). Clusterin ve Fosfor pozitif, güçlü ve istatistiksel olarak anlamlı bir korelasyon gösterdi ($P<0.001$, $r=0.744$).

Bu çalışmanın sonucunda üriner system problemlerinden özellikle böbrek hastalıklarında serum Clusterin düzeylerinin kedilerde analiz edilebilecek bir biyobelirteç olabildiğine karar verildi. Özellikle serum örneklerinde Clusterin seviyelerinin ARF ve CKD'yi tanımlamak için faydalı bir biyobelirteç olabileceği kanaatine varıldı.

Anahtar Kelimeler: Clusterin; CKD; ARF; LUTDs; yarışmalı ELISA.

DETERMINATION OF SERUM AND URINARY CLUSTERIN LEVELS IN CATS WITH RENAL FAILURE

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ABSTRACT

In the present study, the aim of this thesis study was to determine the Clusterin levels in cats with urinary system problems and especially with acute or chronic kidney disease and to investigate whether the relevant parameter is a sensitive biomarker for kidney disease and It is thought that blood and urine levels of Clusterin may be affected in cats with chronic renal failure (CKD), acute kidney failure (ARF) and lower urinary system diseases. In addition to the variations in the levels of the Clusterin parameter in different cases, its correlations with clinical, biochemical, urinary, ultrasonographic and some blood parameters were also investigated. For this purpose, cats including the study and control groups formed the animal material of the research. In the study group, blood samples from a total of 46 cats with chronic renal failure (n=10), acute kidney failure (n=7), lower urinary system disease (n=8), lower urinary tract disease with chronic renal failure (n = 9),
lower urinary tract disease with acute renal failure (n=12) from various breeds brought to Erciyes University Training and Research Hospital. Urine samples from 23 different cats' group were also collected. Control group samples were also taken from 10 healthy cats brought to the same center for general health control. Serum and urine samples obtained from the patients and control groups were analyzed by the competitive ELISA method. The mean serum Clusterin level was found to be 42.87 µg/ml (34.51±57.17) in all sick cats and 36.42 µg/ml (29.08.±48.39) in healthy cats. The mean Clusterin level of the patient group was statistically ($p<0.05$) higher than the mean of the healthy group. The median quantities of Clusterin in urine were found to be statistically considerably lower, 20.59 µg/ml (16.83±25.91) than Clusterin levels measured in patient serum and healthy cat serum ($p<0.001$). Cats with chronic renal failure had statistically significantly higher mean of Clusterin levels (84.79 ± 61.87 µg/ml) than cats with acute kidney failure (38.80 ± 15.18 µg/ml) ($p<0.001$). Clusterin serum mean concentration in cats with LUTD included in the study (23.24 ± 69.12 µg/ml). Serum mean concentration of Clusterin in cats with ARF+LUTD (37.13 ± 10.94 µg/ml). Serum mean concentration of Clusterin in cats with CKD+LUTD (54.75 ± 22.61 µg/ml). Serum Clusterin and BUN levels showed a statistically positive correlation in all patients ($P<0.001$, $r=0.756$),while Clusterin and creatinine levels showed a statistically much stronger and positive correlation ($P<0.001$, $r=0.829$). Clusterin and Phosphorus demonstrated a positive, strong and statistically significant correlation ($P<0.001$, $r=0.744$). As a result of this study, it was decided that serum Clusterin levels can be a biomarker that can be analyzed in cats for kidney diseases. It was concluded that Clusterin levels, especially in serum samples, can be a useful biomarker for identifying ARF and CKD.

Keywords: Clusterin, CKD, ARF, LUTDs, competitive ELISA.

CONTENTS

COMPLIANCE WITH SCIENTIFIC ETHICS i
SUITABILITY FOR GUIDE ii
ACCEPTANCE AND APPROVAL PAGE iii
ACKNOWLEDGMENT v
ÖZET vi
ABSTRACT viii
CONTENTS ix
ABBREVIATIONS xi
LIST OF TABLES xiii
LIST OF FIGURES xiv
1. INTRODUCTION AND AIM 1
2. GENERAL INFORMATION 5
2.1. Chronic Kidney Disease 5
2.1.1. Causes of Chronic kidney disease 6
2.1.2. Pathogenesis of chronic kidney disease in cats 8
2.1.3. CKD-Related Clinical, Biochemical, and Imaging Findings 9
2.2 Feline Lower Urinary Tract Disease 11
2.3 Acute Renal Failure 13
2.4. Biomarkers For Kidney Diseases in Cats and Dogs 14
2.5 The Role of Creatinine in Assessing Kidney Function 14
2.6 Asymmetric Dimethylarginine 15
2.7 Neutrophil-associated lipocalin 16
2.8 The importance of Clusterin in clinical aspect 16
3. MATERIALS AND METHODS 21
3.1. Animal Material 21
3.2. Clinical Examination 22
3.3. Sample Collections 23
3.4. Laboratory Analysis 23

3.4.1. Haematological analysis 23
3.4.2. Biochemical analysis of serum samples 23
3.4.3. Urine analysis 24
3.4.4. Renal Ultrasonographic Examinations 26
3.4.5. ELISA Analysis 26
4. FINDINGS AND RESULTS 31
4.1. Clinical findings 31
4.2. Vital parameters findings 31
4.3. Diseases Diagnosed in Patients 31
4.4. Biochemical Findings 32
4.5. Complete Blood Count Findings 32
4.6. Urine Dip-Stick Examination Findings 33
4.7. Urine Sediment Examination Findings 34
4.8. Renal USG Findings 38
4.9. Clusterin analyses findings 42
5. DISCUSSION 50
6. CONCLUSION AND SUGGESTIONS 58
7. REFERENCES 59
RESUME 70

ABBREVIATIONS

ACE : Angiotensin-Converting Enzyme

AKI : Acute Kidney Injury

AP-1 : Activator Protein 1

ARF : Acute Renal Failure

BUN : Blood Urea Nitrogen

CKD : Chronic Kidney Disease

CLU : Clusterin

DN : Diabetic Nephropathy

EDTA : Ethylenediaminetetraacetic Acid

EDT : Ethylenediaminetetraacetic acid

ELISA : Enzyme-Linked Immunosorbent Assay

ESKD : End Stage Kidney Disease

ET-1 : Endothelin 1

FIV : Feline Immunodeficiency Virus

FLUTD : Feline Lower Urinary Tract Disease

FeLV : Feline Leukemia Virus

FGF23 : Fibroblast Growth Factor 23

GFR : Glomerular Filtration Rate

LVH : Left Ventricular Hypertrophy

NAG : Urinary N-Acetyl-B-D-Glycosaminidase

NFAT : Nuclear Factor Of Activated T-cells

NGAL : Neutrophil Gelatinase-Associated Lipocalin

µL : Micro Liter

RAAS : Renin-Angiotensin-Aldosterone System

RBP : Retinol Binding Protein

SDMA : Symmetric Dimethylarginine

Scr : Serum Creatinine

TGF- β : Transforming Growth Factor β

TGF-β1 : Transforming Growth Factor Beta-1

TIN	: Tubulointerstitial Nephritis
U-Clusterin	: Urinary Clusterin
UPC	: Urine Protein Creatinine
UTI	: Urinary Tract Infection
uCysB	: Urinary Cystatin B
uTP	: Urinary Total Protein
WT1	: Wilims Tumor-1

LIST OF TABLES

Page no

Table 1. Minerals found in the uroliths of dogs and 13

Tablo 2. Division of cats into groups…… 22

Table 3. Preparation of standard solutions for Clusterin analysis…… 28

Table 4. Comparison of physical examination parameters between groups 31

Table 5. The distribution of diseases is presented…… 32

Table 6. Comparison of biochemical parameters between groups…. 32

Table 7. Comparison of complete blood count parameters between groups.....33

Table 8. Comparison of urine dip-stick parameters between groups 34

Table 9. Urine sediment parameters of the patients (urine group)…… 35

Table 10. Comparison of renal USG parameters with reference ranges… 38

Table 11. Comparison of mean/median clusterin levels in patientserum group samples with mean/median clusterin levels of control group. 42

Table 12. Comparison of clusterin levels measured in healthy, patient and urine groups 42

Table 13. Comparison of clusterin levels measured in serum of cats with acute and chronic kidney failure.....43

Table 14. Comparison of Clusterin levels measured in cats serum with urinary tract diseases and acute kidney failure 43

Table 15. Comparison of clusterin levels measured in serum of patient cats with lowe rurinary tract diseases and chronic kidney failure.....43

Table 16. Clusterin levels were evaluated in serum of patient cats with lower urinary tract diseases and cats with mix infection (acute kidney failure with lower urinary tract diseases) 44

Tablo 17. Clusterin levels were evaluated from serum of patient cats with lower urinary tract diseases and cats with mix infection (chronic kidney failure and lower urinary tract diseases)… 44

Table 18. Correlation matrix for clusterin levels…… 45

Table 19. Area Under the Curve……..... 46

LIST OF FIGURES

Figure 1. Hasvet VUC device for dipstick examination ….....25

Figure 2. Formation of sediment (black arrow) following centrifugation processes.....25

Figure. 3. The amino acid sequences of cat clusterin and dog clusterin in the UNIPROT database. ….....27

Figure 4. 4PL fit to standard curve…… 28

Figure 5. ELISA Plate image…….....29

Figure 6. Cat with LUTD revealed Struvite Crystal during examination of urine sediment 35

Figure 7. Cat with urolithiasis revealed calcium oxalate crystal (yellow arrow) and struvite (black arrow) in urine sediment examination.....36

Figure 8. Neoplastic transitional epithelial cell (red arrow) and microorganism (blue arrow) in cat urine sediment (40x) with LUTD.) ….....36

Figure 9. Cats with LUTD showed struvite (black arrow) crystal in urine sediment analysis (40x).....37

Figure 10. Cats with LUTD showed amorphous (black arrow) crystal in urine sediment analysis (40x)….....37

Figure 11. This sonogram (micro-convex transducer, 7.5 MHz) shows presence of urinary bladder stone (red arrow) with shadow (yellow arrow). … ...39

Figure 12. This sonogram (micro-convex transducer, 7.5 MHz) increased length of the left kidney; 6.31 cm,dilatation renal pelvis, increase echogenicity of the cortex 39

Figure 13. This ultrasonography (micro-convex transducer, 7.5 MHz) revealed an enlarged kidney length of 4.82 cm, dilatation of the renal pelvis, and enhanced echogenicity of the cortex in cat with ARF.....40

Figure 14. This sonogram (micro-convex transducer, 7.5 MHz) revealed an enlarged length of the right kidney; 5.43 cm, absence of medulla and cortex differentiation, and presence of anechoic region (fluids) in cats with hydronephrosis.....40

Figure 15. This ultrasonography (micro-convex transducer, 7.5 MHz) revealed an enlarged left kidney length of 4.71 centimetres, dilatation of the

renal pelvis, renal calculi with shadow, and increased echogenicity of the cortex.....41

Figure 16. Representative ultrasonographic images from cat (micro-convex transducer, 7.5 MHz) demonstrating the medullary rim sign (MRS) in the renal parenchyma, which can be observed in kidney disorders 41

Figure 17. ROC Analysis Of CLUSTERIN In The Serum Of The Patient Cats 46

Figure 18. Appearance of the Clusterin levels in patients and control cats using graph. **.....47**

Figure 19. Appearance of the Clusterin levels in cats with ARF and CKD using graph. **.....47**

Figure 20. Appearance of the Clusterin levels in cats with LUTD and CKD using graph 48

Figure 21. Appearance of the Clusterin levels in cats with LUTD and ARF using graph…… **.....48**

Figure 22. Appearance of the Clusterin levels in patients (ARF, CKD, LUTD, HDR, ARF+LUTD, CKD+LUTD) and control cats using graph.....49

1. INTRODUCTION AND AIM

Urinary system diseases are the most common feline problems. In veterinary clinics and hospitals, lower and upper urinary tract diseases of cats, acute-chronic kidney failures and urinary tract infections, which constitute lower urinary system diseases, crystal and stone formations are more frequently encountered.

Chronic kidney disease (CKD) is characterized by a prolonged decline in renal function for at least three months (Finch et al., 2016). It can be caused by either inherited or acquired diseases. Chronic kidney disease is a prevalent issue in small animal medicine, and it typically worsens, producing significant morbidity and mortality in both cats and dogs. Chronic kidney disease affects 13% of the population across both humans and animals. In the general cat population, it occurs around 30% in geriatric cats. Furthermore, CKD affects about 30% of cats over the age of 12 years. It is the second most common cause of death in cats over 5 years old, accounting for 12.1% of all cats (Cannon, 2016). In cats, approximately 10% of cats over age of ten years were reported to be affected by CKD (Finch et al., 2016). Initial kidney injury caused by numerous viral, immune-mediated, congenital, metabolic, neoplastic, traumatic, and obstructive disease processes, and some patterns (e.g., urinary tract blockage) are reversible if treated promptly. Furthermore, renal ischaemia and nephrotoxic exposure are two common causes of renal damage (eg, aminoglycosides, ethylene glycol). Even more nephrons become non-functional as chronic renal disease progresses, and clinical signs of renal failure arise (Thomas and Gallagher, 2007) .

The risk factors may help not only with early detection, but also with understanding disease pathogenesis and creating innovative therapeutics (Bartlett et al., 2010). The development of CKD in cats may occur due to dietary factors. In healthy cats, feeding cats with a commercially available, high-protein, acidifying diet led in hypokalaemia as well as the development of CKD (Finch et al., 2016). Poor body weight, low dietary fiber, different nutritional factors, and *ad libitum* eating are all linked to an increased risk of CKD (Hughes et al., 2002). Cats with weight loss, a disorder affecting body composition, dehydration, a recent history of general anaesthesia, a previous diagnosis of periodontal disease or cystitis, and being male are more likely to be diagnosed with chronic kidney disease (CKD)(Greene et al., 2014).

CKD is a chronic condition which clinical symptoms become more prominent as the disease progresses. Clinical symptoms of chronic kidney disease include increasingly disorganized appearance, changes in their body composition, body weight, and muscular mass.

Polyuria and polydipsia are symptoms that emerge when the kidneys are unable to control the body's water balance. In general, as CKD progresses, the high risk of developing complications increase. The consequences include; hyperphosphatemia, secondary renal hyperparathyroidism, hypokalaemia, anaemia, proteinuria, systemic hypertension, metabolic acidosis, and uraemia (Brown et al., 2016).

The fact that owners typically assign such minor changes in their pets' activity levels, appetites, and coat qualities to "old age" results in these changes being ignored. As azotaemia has progressed, clinical symptoms such as polyuria, polydipsia, dehydration, weight loss, low appetite, halitosis, and occasionally vomiting become more apparent. Ophthalmic, neurological, and/or cardiovascular abnormalities may also occur if hypertension develops as a result of CKD (Cannon, 2016).

Acute renal failure (ARF) is a clinical syndrome that occurs when excretion and renal endocrine functions cannot maintain homeostasis. A sudden decrease is seen in primary renal function due to parenchymal diseases or injuries. It develops over a period of a few hours to a day. In such cases, the glomerular filtration rate is equal to or less than 25%. It is clinically characterized by azotemia and uremia, accompanied

by changes in urine volume (oliguria/anuria). The prognosis depends on the specific etiology and severity of the injury.

Acute kidney injury (AKI), cause decrease in glomerular filtration, urinary output, and tubular function by a sudden decline in kidney function. Azotaemia can develop as a result of these changes, which make it difficult to maintain fluid, electrolyte, and acid-base balance (Monaghan et al., 2012). Toxins, infarction, ischemia, infectious conditions (such as pyelonephritis and leptospirosis), medications, and other factors such as sepsis, hyper viscosity, and hypercalcemia are the most frequent causes of AKI (Mugford et al., 2013).

The clinical manifestations of ARF may include sudden anorexia (not eating), lethargy, vomiting (the vomited material may contain blood), bloody diarrhea, an unusual breath odor, and/or seizures.

Several diseases that affect both the bladder and the urethra of cats are grouped together under the overarching term of feline lower urinary tract disease (FLUTD). The most common symptoms of FLUTD in cats are blood in the urine, increased frequency of urination, with difficulty and discomfort during urinating.

The main causes of FLUTD are urolithiasis, urinary infections, urethral obstructions, idiopathic causes, stress, and other causes like overactivity of the thyroid gland.

Although many urinary system diseases are treatable, treatment options are significantly lower for irreversible and chronic conditions. In such situations, early diagnosis is very important for treating the disease and find out the prognosis of it. Therefore, there is always a need for new and sensitive biomarkers that can identify kidney diseases in the early stages. There are some advantages and disadvantages of kidney injury parameters that are used and known today. Studies on serum or urine clusterin levels as a new biomarker recently investigated in kidney damage in humans, dogs, and rats are still ongoing. Particular renal biomarkers, such Clusterin, are established as prognostic tools for risk prediction, controlling treatment response, and accurate interpretation. To measure the concentrations of Clusterin in different body fluids, such as serum and urine, a number of immunological test techniques have been developed in humans and some animals such as dog and rats in last years.

The urinary Clusterin contains most of the features needed for a biological marker of kidney damage or disease. It is expressed in urine at very low or undetectable levels in healthy kidneys, and its levels rapidly rise in response to renal tissue destruction. Furthermore, it was discovered that urine Clusterin levels fall as a result of kidney injury healing. Because there is a significant risk of irreversible kidney damage development in patients with renal diseases, it is essential to diagnose kidney function abnormalities early using kidney biomarkers in cats.

Clusterin in cats is predicted to serve as a useful biomarker in medical contexts.

The purpose of this study is to measure the levels of Clusterin in cats with renal diseases in order to investigate whether or not this parameter is sensitive for kidney disease.

2. GENERAL INFORMATION

2.1. Chronic Kidney Disease

In both older cats and older dogs, chronic kidney disease (CKD) is the most common renal disorder. It is defined by renal damage that has persisted over than three months and is structurally or functionally impaired in one or both kidneys. Usually, patients with CKD have both loss of structure and function, although the severity of functional impairment doesn't really exactly corresponding loss of structure.

CKD indicates a damage and destruction of renal structure or function that may initially be stable but gradually progresses (Brown et al., 2016). The feline kidney can be affected by a wide range of underlying renal diseases, which are mostly congenital and others of acquired origin, which are also restricted to specific kidney regions. CKD initially does not result in azotaemia (Jepson, 2016).

Despite the fact that it can affect animals of any age, CKD is commonly believed to be a disease of older animals. In general, it is stated that dogs and cats have an incidence of CKD between 0.5 %t and 1.5 % (Brown et al., 2016). The results of an early research revealed that between 15 and 30 % of cats older than 15 years old develop CKD (Jepson, 2016). Chronic kidney disease affects 1 to 3 % of all cats and as much as 35 % of elderly feline populations at referral facilities (Greene et al., 2014). Additionally, according to Kongtasai et al. (2022) the frequency of CKD in cats ranges between 2 and 4 % and rises to 30 to 40 % in cats older than 10 years.

2.1.1. Causes of Chronic kidney disease

The causes are typically unknown and diverse. They had been categorized as congenital and acquired.

Congenital diseases are one of the inherited diseases that have been present at birth. Congenital (existing at birth) or developmental kidney problems are a type of disease in which the kidney may not function properly, may appear abnormal, or may exhibit both impairments. Autosomal dominant polycystic kidney disease, which formerly affected up to 38 percent of Persian and Persian-cross cats, is the most common inherited disease in cats.

Renal amyloidosis in Abyssinian, Siamese, and Oriental cats, as well as glomerular disease in Abyssinian cats, are other congenital feline kidney diseases (Reynolds and Lefebvre, 2013). As well as juvenile renal dysplasia is also one of congenital diseases that can lead to CKD (Aresu et al., 2009).

Acquired causes are commonly seen in cats. As a matter of fact, several acquired CKD cases have been detected in cats. Most frequently primary kidney tumor and renal lymphoma can induce CKD. It has been found that azotaemia affects 16% of cats with CKD and 60% of cats with renal lymphoma (Brice S. Reynolds and Lefebvre., 2013).

Upper urinary tract uroliths had been found in 15% (Bailiff et al., 2008) and 29% (Ross et al., 2006) of cats with CKD in two studies. In cats with ureteral calculi, azotaemia and hyperphosphatemia are common. It's important to mention that 56 % of cats with a small contralateral kidney and 76 % of cats with unilateral ureteral calculi are azotemic, showing that cats with ureterolithiasis frequently have pre-existing renal disease (Kyles et al., 2005).The most prevalent endocrine disorder in older cats is hyperthyroidism, which can be seen simultaneously with CKD. Between 14 and 40 % of hyperthyroid cats develop pre-existing CKD (van Hoek and Daminet, 2009). In cats with hyperthyroidism, the ratio of urine retinol binding protein (RBP) to creatinine concentrations is elevated (van Hoek et al., 2008). Because RBP is easily filtered and usually completely reabsorbed, this rise shows tubular lesions and malfunction and may indicate that hyperthyroidism may lead to renal damage. Both

diseases are frequently accompanied by weight loss, vomiting, polyuria, polydipsia, proteinuria and low urine specific gravity. In 15.3–39% of cats treated for hyperthyroidism, renal disease becomes evident. Hyperthyroidism might cover and even enhance co-existing CKD (Ingrid van Hoek and Daminet, 2009; Van Hoek et al., 2009; Williams et al., 2010).

Proteinuria is not a mediator of the progression of CKD within those patients, but it is an indication of all-cause death in hyperthyroid cats following therapy (Williams et al., 2010).

Contribution of infections to certain CKD cases are possible There has been some evidence connecting feline immunodeficiency virus (FIV) as well as feline leukaemia virus (FeLV) infections to the onset of CKD (Poli et al., 2012). Furthermore, 9.3% of cats with FIV have been reported to have clinical CKD (Brice S. Reynolds and Lefebvre, 2013).

Feline morbillivirus, a previously unknown paramyxovirus was also demonstrated to be connected to tubulointerstitial nephritis (Woo et al., 2012). FeLV infection could well be linked to feline kidney conditions such as chronic renal disease (CKD) and tubulointerstitial nephritis (TIN) (Choi et al., 2020).

Acute kidney injury (AKI) may develope by nephrotoxic medications (such as aminoglycosides, non-steroidal anti-inflammatory medicines, and antineoplastic therapies) or intoxication (such as lily toxicity). Inadequate renal recovery following AKI may result in CKD and speed the progression of end-stage renal disease (Brice S. Reynolds and Lefebvre, 2013).

For several years, healthy cats had been fed with an improper diet (rich in protein and acid, low in potassium) and this has been observed to promote CKD (Brice S. Reynolds and Lefebvre, 2013). *Ad libitum* food and a higher consumption of inorganic mineral content may both raise the chance of developing CKD (Hughes et al., 2002). High salt diets may have a negative impact on kidney function, primarily by causing systemic hypertension. In contrast, in a two-year study on old cats fed with a high salt diet revealed no effect on renal function (Reynolds et al., 2013).

Leptospirosis exposure in cats has been documented, with incidence rates varying from 3 to 35 percent depending on the population and area (Schuller et al., 2015).

2.1.2. Pathogenesis of chronic kidney disease in cats

The most prevalent end consequence of feline CKD appears to be tubulointerstitial fibrosis (Ichii et al., 2011). In most cases, glomerular disease develops as a result of a systemic illness, particularly in neoplastic (e.g., leukaemia, lymphoma), infectious or non-infectious inflammatory conditions (eg. pancreatitis, immune-mediated diseases) (Williams et al., 2010). Tubular damage develops before increases in blood urea nitrogen and creatinine concentrations at early course of the disease (Sato et al., 2002).

In cats with CKD, urine specific gravity drops as plasma creatinine rises, pointing to a direct connection between glomerular and tubular function as feline CKD progresses (Elliott et al., 2003; King et al., 2007).

In addition, chronic hypoxia is frequently present and is linked to higher levels of oxidative stress (Liu, 2011), both of which are likely to have a role in the pathophysiology of feline CKD (Ingrid van Hoek and Daminet, 2009; Keegan and Webb, 2010).

Significant activation of the renin-angiotensin-aldosterone system (RAAS) observed in cats having natural or experimentally induced CKD (Taugner et al., 1996). Angiotensin II causes extracellular matrix to build up through Tranforming Growth Factor Beta -1 (TGF-1) mediated extracellular matrix formation and vasoconstriction of the efferent arteriole (and therefore glomerular hypertension) (Lefebvre and Toutain, 2004). Angiotensin-converting enzyme (ACE) inhibitors have not been demonstrated to enhance histological abnormalities in the kidneys or life durations in cats with CKD, although they do reduce glomerular capillary pressure, the ratio of efferent to afferent arteriolar resistance to blood flow, and proteinuria (King et al., 2006).

In cats with experimental CKD the main changes were in GFR, glomerular pressure and glomerular volume (Brown and Brown, 1995). Since the kidneys have a considerable reserve capacity, azotaemia must be present after at least 75% of renal

function is reduced. For up to a year following the introduction of experimental CKD in cats, GFR found to be stable (Reynolds and Lefebvre, 2013). The course of the disease in cats with spontaneous CKD is unpredictable, with long periods of normal renal function before decompensation (Data, 2003); Chakrabarti et al., 2012).

2.1.3. CKD-Related Clinical, Biochemical, and Imaging Findings

Clinical symptoms of CKD are caused by the retention or loss of nutrients. Clinical indications of chronic kidney disease, such as loss of body condition, muscle mass, and depressive status, are present in many patients, but not in all. The kidneys' failing to maintain the water balance leads in polyuria and polydipsia. There may have been gastroenteritis, ulcerative stomatitis, hyperoxia/anorexia, vomiting and halitosis. Abdominal radiography and ultrasonography both confirm that the kidneys commonly seen as small and irregular in CKD.

On a biochemical tests, hyperphosphatemia, metabolic acidosis and azotaemia are present (urine specific gravity is lower than 1.035 in cats). When used as an indirect measure of GFR, creatinine is more reliable than urea because it goes through glomerular filtration without tube reabsorption and is less affected by factors outside of the kidneys (e.g., intestine protein content, liver function). Whenever there is renal neoplasia, pyelonephritis, or ureteral obstruction, renomegaly may be present. In geriatric cats with age-related muscle wasting or when muscle mass steadily falls as CKD progresses, muscular mass is critical (Lees, 2004). Renal disease progression has been linked to hyperphosphatemia in humans due to decreased in renal excretion (Zoccali et al., 2011).

Many of the processes that have been linked to the progression of renal diseases may also be affected by phosphate, including cell necrosis, the effects of aging, and the oxidative stress. It has been suggested that elevated levels of extracellular phosphate, stimulate the RAAS, the phosphate excretion attenuates with declining kidney function. Resulting FGF23 elevation counteracts the phosphate retention by downregulating NaPi-2’a/c in the kidney, but also directly promotes LVH via FGFR4-calcineruin-NFAT signaling. FGF23 induces hypertension by activation of the RAAS and NCC expression as a result, enhance the synthesis of profibrotic mediators (Jepson, 2016).

Additionally, certain cats may get bacterial urinary tract infection (UTI), nonregenerative anaemia, hypoalbuminemia, dyslipidaemia, and hypokalaemia (which is more prevalent in cats than in dogs).Furthermore, between 40% and 80% of patients develop arterial systemic hypertension (Bartlett et al., 2010).

Proteinuria, is related to progression when canine and feline CKD is accompanied with proteinuria (Jacob et al., 2005; Syme et al., 2006). Although proteinuria is often conceived of as a symptom of glomerular damage, it actually seems to be nephrotoxic even in the absence of forceful primary glomerular disease (Elliott and Syme, 2006). CKD and systemic hypertension commonly coexist, The pathogenesis of hypertension in cats with CKD is multifactorial and includes aspects such as; reduced sodium excretion, RAAS activation, stimulation of the sympathetic nervous system, arterial structural alterations, endothelial dysfunction, and oxidative stress (Reynolds and Lefebvre, 2013). In addition to increasing the risk of secondary end-organ damage, systemic hypertension can decrease renal function by inducing proteinuria and glomerular hypertension (i.e., ocular, neurological and cardiac) (Acierno et al., 2018).

In addition, CKD is the most prevalent cause of hypokalaemia in cats. Cats with severe hypokalaemia are three and a half times more likely to develop CKD (Reynolds and Lefebvre, 2013). Potassium loss from CKD may contribute to the development of renal disease and the worsening of the patient's condition.

In CKD cats, hyperkalaemia is unusual (prevalence 5.8 percent) (King et al., 2006) ; King et al., 2007), but only in oliguric CKD at the end stage (Reynolds and Lefebvre, 2013).

Cats with CKD typically develop a normochromic, normocytic, hypoproliferative anaemia, particularly during the last stages of the condition (Chalhoub et al., 2011). Anaemia occurs in 30–65 percent of cats with chronic kidney disease (Chalhoub et al., 2011). As the degree of CKD worsens, so does the Pocked Cell Volume. The peritubular fibroblast type-1 interstitial cells are the primary source of erythropoietin deficiency in patients with chronic kidney disease. Anaemia adversely affects the quality of life of cats with CKD (Chalhoub et al., 2011).

The incidence of metabolic acidosis varies from 22–88 percent according on the severity of CKD (Elliott et al., 2003). Late in the course of CKD, metabolic acidosis usually ensues (Data, 2003). Low plasma bicarbonate and chloride concentrations lead to an anion gap increase (Elliott et al., 2003), with hypochloraemia developing before acidosis occurs (Data, 2003). Hypokalaemia can cause metabolic acidosis (Dow et al., 1990), however hypokalaemic cats are normally capable of maintaining normal venous blood pH and bicarbonate concentrations in the bloodstream (Data, 2003). In CKD, hydrogen ion excretion by renal ammoniagenesis (RA) may also be compromised (Data, 2003). Gastric hyperacidity or vomiting can cause a loss of chloride from the gastrointestinal system and leading to hypochloraemia (Data, 2003). In renal patients, metabolic acidosis can lead to a variety of medical conditions, including anorexia, vomiting, fatigue, and malnutrition (Brice S. Reynolds and Lefebvre, 2013).

2.2 Feline Lower Urinary Tract Disease

Diseases of the bladder and urethra in cats are often referred to as feline lower urinary tract disease (FLUTD). As a result of the restricted ways in which the urinary tract can react to injury, the clinical symptoms are typically not diagnostic. Many other diseases and health problems can cause FLUTD symptoms, although idiopathic cases make up the great majority of reported cases. The causes of FLUTD without obstruction 65% of cases of cystitis are due to idiopathic causes that do not include obstruction. Uroliths account about 15% of all stones. Ten percent due to abnormal anatomy, cancer, or other conditions Disordered behavior, less than 10% Infection due to bacteria. Causes of obstructive FLUTD Obstructive idiopathic cystitis are 29%, 59% of patients treated with a urethral plug. Uroliths account of 10% of all kidney stones. Bacterial infection + uroliths, 2% (Syme, 2012).

Dysuria, pollakiuria (increased frequency of urination), haematuria, urethral obstruction, periuria (inappropriate urination), and/or behavioral changes and loss of litter-box training are common symptoms of feline lower urinary tract disease.

Urolithiasis is a condition in which stones, or calculi, form in the urinary tract. Uroliths can have a wide range of mineral compositions, the most prevalent of which being struvite and oxalate. Many researchers have spent the last few years working

on the development of diets that can aid in the dissolving of struvite stones. While this has helped reduce the prevalence of struvite urolithiasis, it has led to an uptick in oxalate urolithiasis(Syme, 2012).

Idiopathic cystitis in cats (FIC) rarely is a cause identified for FLUTD, but in the majority of instances there is no apparent trigger. After 30 years of study, scientists still haven't pinpointed what triggers. A current theory proposes that abnormalities in the relationship between the neuronal supply to and from the bladder, the protective glycosaminoglycan (GAG) layer lining the bladder, and chemicals in the urine may contribute to the development of FIC. Some researchers have hypothesized that FIC is analogous to interstitial cystitis.

Crystalluria is a condition in which the urine contains crystals. This happens when the body has an excess of various electrolytes. Despite the fact that it is not a diagnostic indication in cats, crystalluria is the strongest predictor of return of uroliths in humans (Okafor et al., 2019). More than 97% of the urinary tract stones (nephroliths and ureteroliths) that are submitted for analysis have been removed from the lower urinary tract (Osborne et al., 2009). Dogs and cats that have bladder stones may present with symptoms of the lower urinary tract (such as pollakiuria, haematuria, and stranguria), or they may present with urethral obstruction due to the development of the condition (usually only in males) (Syme, 2012).

Cats with disorders of the lower urinary tract frequently have struvite crystals (magnesium ammonium phosphate hexahydrate) in the urine. Struvite has emerged as the most prevalent crystal found in feline urethral plugs during the past decade (Osborne et al., 2009). Stones composed of struvite are relatively common in canines and felines, accounting for nearly half of all the stones that have been sent in for examination (Syme, 2012).

In 2007, 41.3 % of the stones presented from dogs and 40.8 % of the stones received from cats were calcium oxalate stones (Osborne et al., 2009). But in last years it is determined that struvite crystals rate is higher than calcium oxalate in both cats and dogs (Syme, 2012).

Table 1. Minerals found in the uroliths of dogs and cats (Syme, 2012).

Struvite	39.0	48.6
Calcium oxalate	41.3	40.8
Urate	5.0	4.9
Cystine	1.1	<0.1
Mixed	2.8	0.7
Compound	9.1	3.2

2.3 Acute Renal Failure

Acute renal failure (ARF) is a commonly recognized disease state that necessitates prompt and aggressive management in cats. To provide the most effective and timely therapy, a detailed grasp of the pathophysiologic processes underlying ARF and familiarity with the most prevalent etiologies are needed. Possessing this information will also permit a more accurate prediction and increase the chances of a favorable outcome.

Although the actual origin of ARF is unknown in around one-third of feline patients, it is essential to investigate the etiology due to its potential impact on treatment and prognosis. Toxins are the leading cause of ARF in approximately fifty percent of feline patients with ARF, Cats may be exposed to nephrotoxic compounds such as lilies, non-steroidal anti-inflammatory medications (NSAIDs), ethylene glycol (EG), aminoglycosides, doxorubicin, vitamin D, and dietary pollutants including melamine and cyanuric acid. Other probable causes of ARF include ischemic injuries, upper urinary tract infection (pyelonephritis), neoplasia, chronic ureteral or urethral blockage, and sepsis (Monaghan et al., 2012).

Damage to peritubular capillary endothelial cells results in an increase in vascular responsiveness to vasoconstrictive agents and a decrease in vascular reactivity to vasodilating agents, resulting in a worsening of ischemia and hypoxia. Damaged endothelial cells upregulate the surface expression of P- and E-selectin as well as ICAM-1 (intercellular adhesion molecule-1). These are mediators of the interaction between endothelial cells and leukocytes, and their production increases adhesion and activation of leukocytes. This mechanism promotes the inflammatory cascade, leading to increased vascular congestion and ischemia (Monaghan et al., 2012).

2.4. Biomarkers For Kidney Diseases in Cats and Dogs

A biomarker is a measurable and repeatable physical, functional, or biochemical indicator of a physiological or pathological process that can be used for diagnostic or prognostic purposes. Biomarkers can be classified as functional or physical, depending on how they function. A biomarker's current function is quickly increasing further than the diagnosis, progression of the disease and regression (Yerramilli et al., 2016). An ideal biomarker for the diagnosis of kidney disease would be one that was both non-invasive and specific, in addition to being sensitive enough to enable early diagnosis of the condition. In addition, it should make it possible to specify the extent or severity of the disease, and it should be suitable for monitoring the progression of the disease, as well as helpful in determining the location of the injury and the outcome of treatment (Kovarikova, 2015). Obtaining biomarker measurements from a reference laboratory or point-of-care test should be simple, affordable, and widely available (Cobrin et al., 2013).

The most accurate and sensitive test for renal dysfunction is the evaluation of glomerular filtration rate (GFR) (Finch, 2014). Estimating the clearance of an endogenous or exogenous filtration marker is a direct way to figure out the GFR (Finch, 2014). Direct GFR measurement is still primarily used as a research tool, despite the limited sampling techniques that have been developed to improve the practical use of GFR measurement in cats (Ghys et al., 2016).

In some cases, protein biomarkers are not organ-specific because they are secreted by multiple tissues (Rosenberg and Silkensen, 1995). Clusterin messenger RNA, for example, is highly concentrated in the liver, stomach, brain, and testes, but is present in all animal tissues. Cells of the immune system, the hepatocyte, the adipocyte, the epithelial lining of the liver, lung, colon, and renal tubules all express and secrete Neutrophil-associated lipocalin (NGAL) under certain pathologic conditions (Chakraborty et al., 2012).

2.5 The Role of Creatinine in Assessing Kidney Function

100 years ago, serum creatinine was the standard test for kidney function and disease (Baxmann et al., 2008). It is muscle breakdown products which correlate with muscle mass. In cachectic, low-muscled animals, kidney function is overestimated, while in

heavily muscled animals, kidney dysfunction may be misdiagnosed. Creatinine metabolism must be steady to reflect kidney function, which makes it difficult to find small losses in kidney function. Human patients show gender, age, and race differences. Due to these limitations, kidney function may decline severely before creatinine levels change (Dalton, 2010). Due to false-positives (true tubular injury but no noticeable change in serum creatinine) or false-negatives (absence of true tubular injury but increases in serum creatinine level) brought on by pre-renal problems or any of the numerous confounding factors, the dependence on creatinine puts the new biomarkers at risk for poor accuracy and performance.

2.6 Asymmetric Dimethylarginine

Analogous of L-arginine, asymmetric dimethylarginine (ADMA) is a metabolic byproduct found in the bloodstream. When present in high concentrations, ADMA inhibits the production of nitric oxide (NO), which in turn reduces endothelial function and contributes to kidney impairment (Vallance et al, 1992). There are many factors that influence renal function, but nitric oxide plays a crucial role. Two studies suggest that elevated levels of ADMA are the strongest risk predictor involved in the development of progressive nephropathies. Elevated ADMA consistently predicted a more rapid decline in renal function, lending credence to the idea that ADMA itself may be a causative factor in renal damage by causing glomerular hypertension, endothelial damage, salt accumulation, and cellular senescence as concluded by two different studies (Fliser et al., 2005; Ravani et al., 2005).

Human urine was used to isolate the first samples of symmetric dimethylated arginine (SDMA) and asymmetrical dimethylated arginine (ADMA) (Yerramilli et al., 2016) . Numerous studies in various clinical settings have proven SDMA to be a highly accurate and sensitive biomarker of kidney function. In a prospective study of cats, the relationship between muscle mass, creatinine and SDMA levels was examined. SDMA levels increased, indicating that it is unaffected by changes in muscle mass and truly reflecting decreased renal function (Hall et al., 2014). SDMA has recently been approved as a biomarker for renal function in dogs and cats by the International Renal Interest Society (IRIS).

2.7 Neutrophil-associated lipocalin

Neutrophil-associated lipocalin (NGAL) is a 24-kDa protein that binds iron-containing ligands. It is also known as 24p3, SIP24, lipocalin 2, or siderocalin (siderophores) (Devarajan, 2008). It was first observed in human neutrophils. Skin, alveolar and oral mucosa, adipose tissue, proximal and distal tubules, and other tissues contain NGAL (Chakraborty et al., 2012). It is strongly upregulated in neoplasia and in injured epithelial cells of the kidney, colon, liver, and lung. Multiple canine studies have shown that elevated NGAL levels earlier than increased serum creatinine levels in AKI (Segev et al., 2013). Common inflammatory diseases like eczema, periodontitis, and ulcerative colitis, as well as metabolic disorders like obesity and type II diabetes, are associated with significant upregulation of NGAL. The cancers of the skin, thyroid, breast, liver, and stomach are all lead to overexpress of NGAL (Chakraborty et al., 2012). Serum creatinine level, glomerular filtration rate (GFR), and proteinuria all appear to have strong correlations with urinary NGAL concentrations.

2.8 The importance of Clusterin in clinical aspect

In 1979, researchers found and isolated Clusterin, ApoJ, SPG2, TRPM-2, or CLU in the testis of a rat (Hogg and Embery, 1979). Clusterin was given its name because of its ability to aggregate blood cells in vitro (Blaschuk et al., 1983). One of the most common and important cellular components which is found in almost all fluids and parts of the intracellular matrix.

Various isoforms can be generated from the initial protein precursor, such as a small nuclear isoform (49kD), a medium-sized isoform that remains in the cytosol and mitochondria, and a large nuclear isoform The Golgi apparatus is able to cleave large glycosylated proteins (53kDa) and secrete them in the form of α-β heterodimer that can be found outside the cell (75–80kDa). All of them serving distinctly different purposes (Rohne et al., 2016). It is expressed from a diverse range of tissues and plays a role in a variety of physiologic processes, such as maturation of sperm, transportation of lipids, the inhibition of complement, remodeling of tissue, recycling of membranes, stabilization of stressed proteins, and prevention of apoptosis. It is also expressed from a wide range of tissues (Blaschuk et al., 1983).

Because of its chaperone activity, the secreted isoform of clusterin was initially linked to Alzheimer's disease. Protein misfolding and cellular debris in the interstitial compartment around tissue cells are important targets for Clusterin. So, traditionally, it has been thought to protect the nerves (Iłżecka et al., 2019). Clusterin's protective action here is designed to help cells survive.

Despite the fact that kidney tubular injury causes an increase in urinary Clusterin concentrations, serum Clusterin concentrations are impacted by a variety of physiological and pathophysiologic processes, such as sperm development, Neurodegenerative disorders, atherosclerosis, and cancer (Patel et al., 2004); Rodríguez-Piñeiro et al., 2006).

Clusterin has also been shown to have a role in protecting against the oncological discomfort that results from cancer treatment in recent years. In addition to this, it has also been suggested as a possible option for the treatment of other kinds of pain, such as neurogenic and inflammatory pain (Oh et al., 2013). It has been suggested that Clusterin may play a role in inflammatory processes including obesity, where it has been linked to the management of food intake (Gil et al., 2013). It has been proposed that the presence of this protein may be related to the development of addictive behaviours (Pallardo-Fernández et al., 2020). A protective role in heart disease has also been asserted in recent years, as evidenced by numerous studies (Bass-Stringer et al., 2020).

The International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the United States Food and Drug Administration (FDA) both use urinary Clusterin as a kidney biomarker in drug development and toxicity testing. Clusterin, previously found as a marker of kidney injury in a differential expression experiment involving healthy and diseased dogs, was also identified (Yerramilli et al., 2016) .

Clusterin levels in different body fluids, such as plasma, serum, and urine, can be determined using a number of immunoassays that have been created and put on the market. The majority of the requirements for a kidney disease or injury biomarker are achieved by urinary Clusterin (Yerramilli et al., 2016). When the kidneys are injured, Clusterin is elevated and secreted into the urine (Rosenberg et al., 2002). A

broad indicator of renal damage, U-Clusterin has no specific nephronal site (Dieterle et al., 2010). Patients with diabetic nephropathy (DN) and streptozotocin-induced diabetic mice both had higher levels of glomerular Clusterin expression, which was discovered to be co-localized with the podocyte marker WT1, demonstrating that Clusterin is expressed in podocytes (He et al., 2020). Results show that glomerular Clusterin is increased in DN and this increase in Clusterin expression may protect podocytes from oxidative stress-induced apoptosis, giving a possible novel treatment target for DN and other kidney diseases (He et al., 2020). In a healthy kidney, it is expressed in urine at negligible to undetectable quantities, but when a kidney is injured, its levels rise noticeably. In addition, it has been discovered that urine Clusterin levels fall as a result of kidney injury healing (Yerramilli et al., 2016).

Polycystic kidney disease with tubular damage, but not focal glomerulosclerosis, causes an increase in Clusterin in the urine of rats, according to Hidaka et al. (2002), which this study suggested that the detection of U-Clusterin may be useful in distinguishing tubular proteinuria from glomerular proteinuria.

In terms of diagnosing proximal tubular injury, urine Clusterin outperformed BUN and SCr, while urinary total protein, cystatin C, and 2-microglobulin exceeded BUN and SCr in terms of detecting glomerular injury (Dieterle et al., 2010).

A number of kidney disorders, particularly posturethral obstruction, have been linked to an increase in the synthesis of Clusterin(Pearse et al., 1992), during an injury caused by ischemia-reperfusion (Rosenberg and Paller, 1991).

García-Martínez et al. (2012), used a leishmaniasis model of kidney disease to test U-clusterin as a biomarker of renal impairment in dogs with varying stages. They also validated a species-specific enzyme immunoassay (EIA) for measuring U-clusterin in dogs. Urine Clusterin concentrations were found to be higher in dogs with renal impairment, and the urinary Clusterin-creatinine ratio could be employed as an early indicator of chronic kidney disease.

Thus, Wu et al. (2018) speculated that, indicator of tubulointerstitial renal lesions, urinary Clusterin is a newly discovered biomarker that exhibits a correlation with and best predicts end stage kidney diasease (ESKD).

Hidaka et al. (2002), were established an ELISA to quantify urinary clusterin levels in different renal injuries and to evaluate the clinical use of Clusterin concentrations as a clinical marker. The findings suggested that the concentration of Clusterin in the urine might therefore be used as a useful marker for determining the extent of tubular injury. In addition to this, Clusterin has the potential to assist in distinguishing between tubular and glomerular types of proteinuria.

In a study, Correa-Rotter et al. (1998) investigated the expression of Clusterin in a model of glomerular damage that is not mediated by complement, which is puromycin aminonucleoside nephrosis. Induction of Clusterin production in tubular epithelial cells was seen to take place in the kidneys of nephrotic rats, as demonstrated by their results. The expression of Clusterin occurs before the development of tubulointerstitial disease and is thought to be a reaction to the proteinuria (Correa-Rotter et al., 1998).

Shannan et al. (2006), showed that understanding of the significance of CLU in a variety of physiological activities, one of which is the growth of tumors, and explain the implications of this finding to the treatment of cancer in the future. According to the preliminary findings, therapeutic approaches targeting CLU have the potential to be useful in the treatment of cancer. However, these tactics should take precautions to ensure that nucleus CLU does not become extinct.

Zhou et al. (2014), evaluated of the performance of four urine biomarkers, including NGAL, Clusterin, NAG, and uTP, related to histopathology and routine clinical chemistry parameters in the model of acute kidney injury produced by gentamicin in beagle dogs. Clusterin proved to be the most sensitive biomarker for the identification of gentamicin-induced renal proximal tubular damage.

In another study, urinary levels of uCysB and uClusterin, as well as GFR alternative indicators (sCr and SDMA) and UPC, were the main focus of this investigation in dogs toxicated by Vipera berus. In addition, to see if the severity of clinical manifestations of toxication was related to the concentration of AKI indicators. Using this information in a therapeutic setting could enhance in the treatment of these pets and improve outcomes. In envenomated dogs, urinary cystatin B concentrations were considerably greater than in controls at all-time points. Clusterin

concentrations were not substantially different from controls in the urine at any point (Harjen et al. 2022)

3. MATERIALS AND METHODS

The research was conducted at the Erciyes University Faculty of Veterinary Medicine department of internal medicine. Chronic renal failure, acute renal failure, and lower urinary tract disorders were diagnosed, following the owners' willingness to participate in the study for the aim of treatments at the Erciyes University teaching and Research Hospital. Blood and urine samples collected from patient and healthy cats which came for health check-ups.

The study was approved by Erciyes University Animal Experiments Local Ethics Committee (ERU HADYEK) for animal experimentations (committee decision number: 21/199).

3.1. Animal Material

In this research, blood and urine samples were taken from all cats of various breeds allocated to the study group and the control group. Haematological, biochemical, and urinary analysis were carried out in these samples. In the study group, blood (n= 46) and urine samples (n= 23) were collected form cats with chronic kidney failure, acute kidney failure, and lower urinary tract diseases admitted Erciyes University Veterinary Teaching and Research Hospital. Blood samples were also collected from 10 healthy cats as control group we did not collect urine samples from healthy cats because we did not have permission from the owners.

Clinical symptoms of acute, chronic kidney failure, and lower urinary tract diseases were anorexia, vomiting, polyuria, polydipsia, dysuria, anuria, lethargy and dehydration were taken as initial inclusion criteria in the study. Case definitions were as follow.

Cats with acute renal failure (ARF);with symptoms less than seven days, azotaemia (serum creatinine level greater than 2.5 mg/dL and blood urea nitrogen level greater than 35 mg/dL), urine specific gravity lower than 1.025, anuria, or an increase in serum creatinine concentration despite fluid therapy and normal hydration; palpable large or normal-sized kidneys (Worwag and Langston, 2008).

Cats with chronic kidney disease (CKD) included in the study, the kidneys frequently appeared as small and irregular on ultrasonography examination. Biochemically, azotemia with excessively dilute urine (urine specific gravity low than 1.035 in felines) and hyperphosphatemia and anemia was present (Kovarikova, 2015).

Cats with LUTD were included in the study Urine dipstick pads used to detect white blood cell (WBC) were frequently positive, in urine sediment examination, urine contained >5 WBCs per high-power field, crystals with stone or urethral plugs in microscobic urine sediment examinations demonstrated (Hostutler et al., 2005).

Cats with neurological, respiratory, skin, and other diseases were excluded the study.

Haematological, biochemical and urine examinations were performed on the same day of sampling.

Table 2: Division of cats into groups

Animals		**Distribution**	**Number of Animals for Each Group**
Cats	**Groups**	Group I: Patient Cats (Serum)	46
		Group II: Patient Cats (Urine)	23
		Group III: Healthy Cats (Serum)	10

Patient cats include chronic kidney failure, acute renal failure, lower urinary tract diseases. Patient cats (serum) for detection Clusterin levels in serum samples. Other Patients cats (urine) for dectection Clusterin levels in urine sample from differenet cat group. Third group is from healthy cats (control group) for detection Clusterin levels in their serum samples.

3.2. Clinical Examination

Sick cats were admited to the Erciyes University, Faculty of Veterinary Medicine Teaching and Research Hospital for the first time with the complains of loss of appetite, depression, vomiting, polyuria, polydipsia, dysuria, and anuria. Clinical examinations such as body temperature, heart rate, respiratory rate and laboratory

assessment including haematology, biochemistry and urine analyses (only on 23 patient cats) were performed in these patient cats

Healthy cats were routinely examined for check-ups at the hospital. Laboratory analysis (hemogram, biochemistry and urine tests) were included on these check-ups. Healthy cats were considered as control group in this thesis

3.3. Sample Collections

Blood samples were collected from the cephalic vein into tubes with an anticoagulant (BD, K3EDTA, 3.8 ml for haematology and into tubes without anticoagulants (Hemolab BD, Supersonic transport (SST) 6 ML) for biochemical analysis. Urine samples were collected by either cystocentesis or catheterization for urinalysis (dipstick test and urine sediment examination). The ultrasonography examinations of the kidneys and urinary tract were also conducted on patient groups.

3.4. Laboratory Analysis

Complete blood count was performed on a cell counter within an hour of sampling urine and serum samples for biochemical analyses were examined within 1-2 hours at Erciyes University Veterinary Practical and Teaching hospital in the normal room temprature. Blood samples were centrifugated at 3000 rpm for 15 minutes on a device (Hettich, Zentrifugen), and serum was hervested and stored at at -80 ^{o}C until analyses. The urine was analyzed with a dipstick test before being centrifuged at 3000 rpm for 5 minutes in order to examine sediment. After urine centrifugation, the supernatants of the urine samples were also stored at -80 ^{o}C until analyses.

3.4.1. Haematological analysis

Completet blood count was performed using Mindray BC2800 complete blood count device after 3 minutes of homogenization on a roller (Hemolab).

3.4.2. Biochemical analysis of serum samples

Clusterin was assayed using a commercial ELISA. A Randox Monaco brand completely automatic biochemistry analyzer was used to determine BUN, Creatinine, and inorganic phosphorus levels of all cats.

3.4.3. Urine analysis

Dipstick strips (Hasvet VUC) were used for urinalysis (Fig. 1). Preparation of urine sediment (Fig. 2) was performed to identify cells, casts, crystals, and/or microorganisms. Urine samples were centrifuged (Hettich, Zentrifugen) for 5 minutes at 3000 rpm to obtaine sediments. The supernatant was collected and mixed by turning the bottle upside down. 5 ml of urine was transferred to a centrifuge tube with a conical tip using a disposable plastic pipette. Each urine supernatant was split into two Eppendorf tubes and refrigerated at -80 ^{o}C until analysis.

A drop of the remaining sediment was placed on a microscope slide using a pipette. A coverslip was placed over the sample. The covered slide was placed on the microscope and examined using the low power (x10) lens followed by the high power (x40) lens in order to determine cells, casts, crystals, and/or microorganisms. The findings were gradeded as grade zero means no cells, grade one means 1-5 cells, and grade two means more than six cells in a high-power field. Pyuria (>six white blood cells/high power field), haematuria (>six red blood cells/HPF), and bacteriuria were also noted as reported by Yadav et al., (2020).



Figure 1. Hasvet VUC device for dipstick examination.



Figure 2. Formation of sediment (black arrow) following centrifugation processes.

3.4.4. Renal Ultrasonographic Examinations

Hair was clipped and an acoustic coupling gel was applied to the skin on the abdomen. For longitudinal imaging, the transducer was positioned parallel to the animal's long axis.In this study, each ill cat was examined using an Mindray DC-N3 ultrasound system with a convex (5.0–7.5 MHz) transducer. Kidneys and bladder were checeked in order to detect the urinary bladder wall thickness, presence of mineralization in the bladder, determine the thickness of the renal cortex and the medulla, as well as the presence of hyperechogenicity on the renal cortex. Normal cat kidney measurements were in the range of 3.0 to 4.3 cm, a cortical thickness of 0.82 ± 0.14 cm and a medullary thickness of (0.59 ± 0.06) cm (Debruyn et al., 2012).

3.4.5. ELISA Analysis

A commercial Dog ELISA Clusterin Kit t was used for analyses. Since no company produced a Clusterin ELISA kit for cats, the clusterin ELISA kit for dogs was used instead. Because clusterin shares a high degree, approximately 99%, of homology of, the amino acid sequences of both cat and dog as shown in the UNIPROT database (Fig. 3).

Cat Clusterin aminoacid sequences

>sp|A0M8S7|CAV1_FELCA OS=Felis catus OX=9685 GN=CAV1 PE=3 SV=1

MSGGKYVDSEGHLYTVPIREQGNIYKPNNKAMAEEINEKQVYDAHTKEIDL
VNRDPKHLNDDVVKIDFEDVIAEPEGTHSFDGIWKASFTTFTVTKYWFYRLL
SALFGIPMALIWGIYFAILSFLHIWAVVPCIKSFLIEIQCISRVYSIYVHTFCDPF
FEAVGKIFSNIRINMQKEI

Dog Clusterin aminoacid sequences

>sp|P33724|CAV1_CANLF OS=Canis lupus familiaris OX=9615 GN=CAV1 PE=1 SV=1

MSGGKYVDSE GHLYTVPIRE QGNIYKPNNK AMAEEMSEKQ VYDAHTKEID LVNRDPKHLNDDVVKIDFED VIAEPEGTHS FDGIWKASFT TFTVTKYWFY RLLSALFGIP MALIWGIYFAILSFLHIWAV VPCIKSFLIE IQCISRVYSI YVHTFCDPFF EAVGKIFSNI RINMQKET

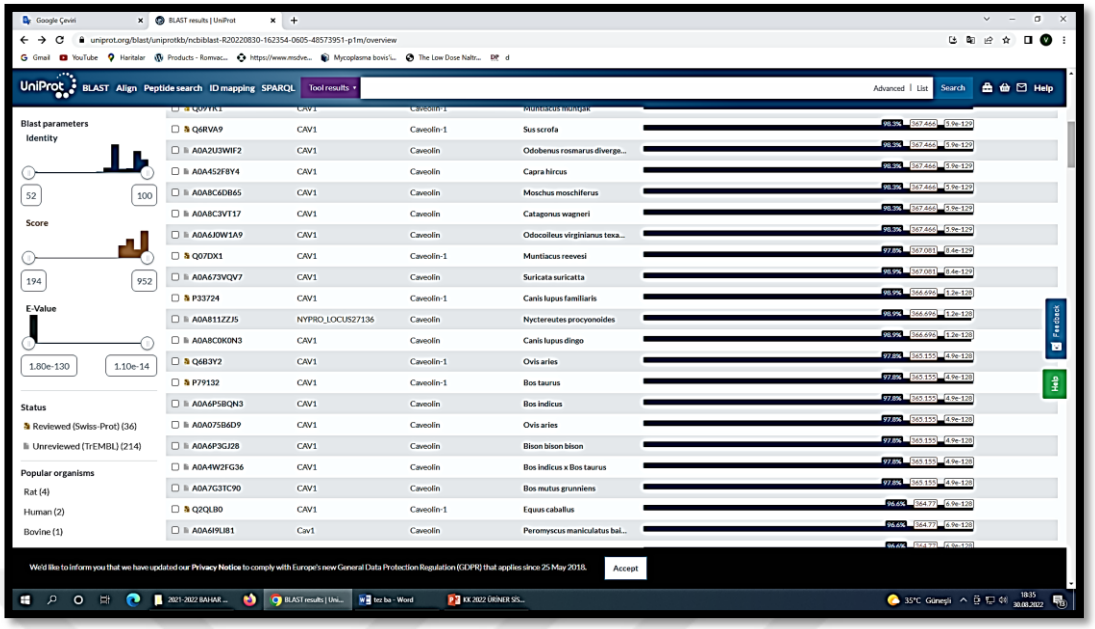


Figure 3: The amino acid sequences of cat Clusterin and dog Clusterin in the UNIPROT database.

ELISA was carried and evaluated as instructed by the manufacturer. The optic densities were obtained at 450 nm by using Biotek ELx800 ELISA reader. The standard curve and Clusterin concentration in the urine and serum samples were determined using a 4-parameter logistic curve-fitting program.

Clusterin analysis

Before the test, samples were prepared by diluting the serum and the supernatant of the urine samples with sample diluent (1:2500). The suggested 2500-fold dilution was achieved by adding 2 µl sample to 98 µl of sample diluent. Complete the 2500-fold dilution by adding 5 µl of this solution to 245µl of sample diluent. The standard was prepared by diluting it with 1 mL of sample diluent. The dilution yields a stock solution of 500 ng/l. Each tube (S0-s6) had 150 µl of sample diluent pipetted into it. The stock solution had been used to create a 2-fold dilution series. HRP-conjugate preparations, 10 µl of HRP-conjugate + 990 µl of HRP-conjugate diluent.

Table 3. Preparation of standard solutions for Clusterin analysis.

Clusterin Concentrations	Standard Number	Serial Diluations
500 ng/ml	Stand. No. 7	150 µl Original Standard + 150 µl Standard Dilution Solution
250 ng/ml	Stand. No.6	150 µl Standard 7 + 150 µl Standard Diluent
125 ng/ml	Stand. No. 5	150 µl Standard 6 +150 µl Standard Diluent
62.5 ng/ml	Stand. No. 4	150 µl Standard 5 +150 µl Standard Diluent
31.25 ng/ml	Stand. No. 3	150 µl Standard 4 + 150 µl Standard Diluent
15.625 ng/ml	Stand. No. 2	150 µl Standard 3 + 150 µl Standard Diluent
7.813 ng/ml	Stand. No. 1	150 µl Standard 2 + 150 µl Standard Diluent
0 ng/ml	Stand. No. 0	150 µl Standard Dilution Solution

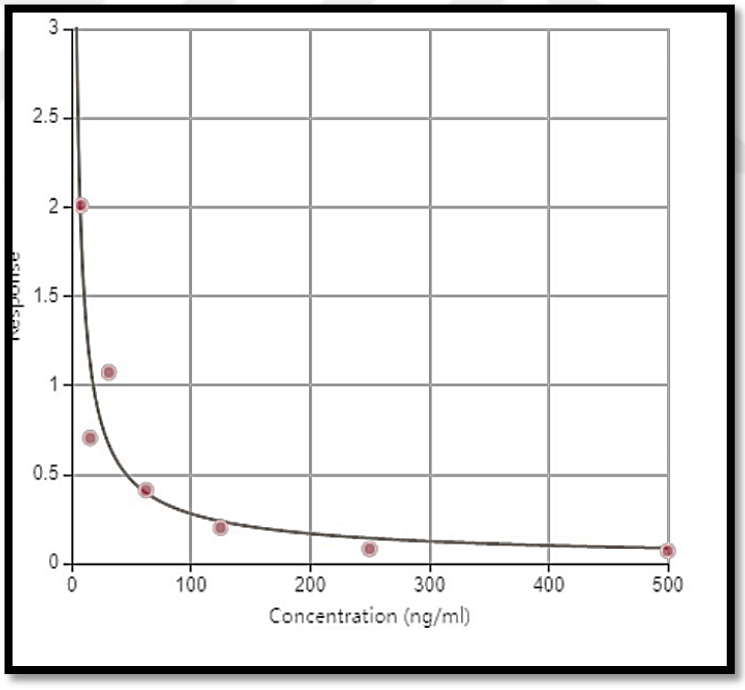


Figure 4. 4PL fit to standard curve (R^2: 0,89, $y = d + \frac{a-d}{1+(\frac{x}{c})^b}$)

Procedure for Analysing

Clusterin analysis was carried out in accordance with the instructions contained in the kit (CSB-E13770c/CUSABio).

1. Two blank wells had been set up with no solution.
2. Each well received 50 µl of standard and sample, followed by 50 µl of HRP-CONJUGATE. The microtiter plated had been incubated for 60 minutes after adding adhesive strips.
3. Each well had been aspirated and washed five times with 200 µl of wash buffer. The plate had been reversed and blotted with clean paper towels after the last wash.
4. 90 µl of TMB substrate being added to each well and incubated for 20 minutes at 37 ^{o}C in the dark.
5. Each well received 50 µl of stop solution.
6. The results have been obtained at 450 nm using the Biotek ELx800 ELISA device.

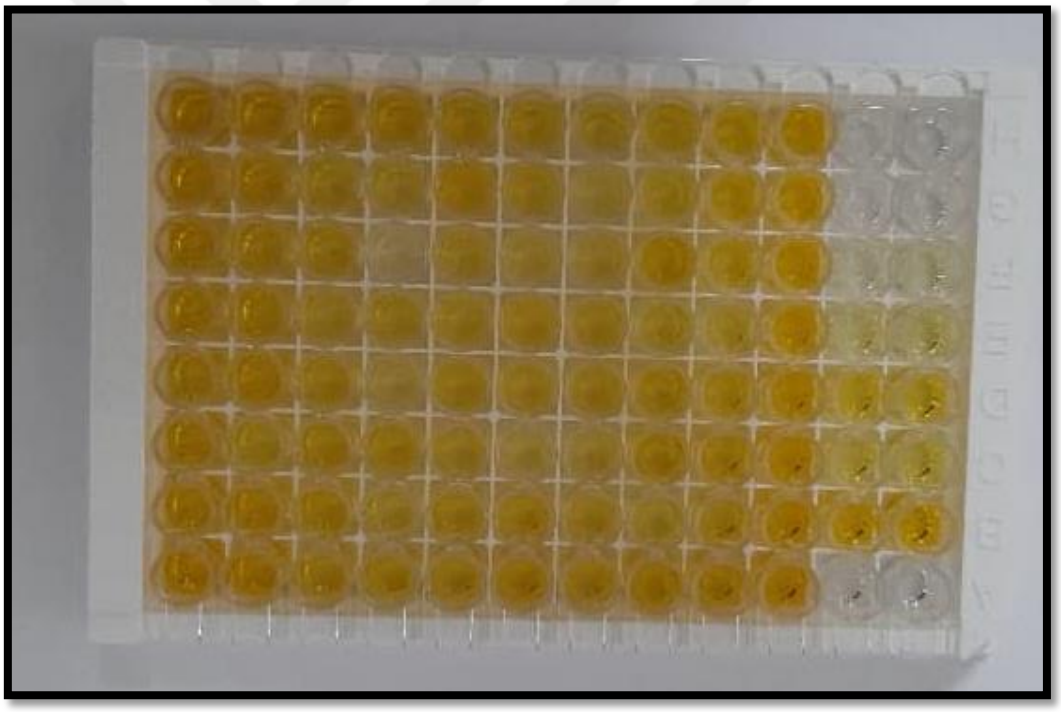


Figure 5. ELISA Plate image

3.5. Statistical Analyses

The IBM-SPSS for Windows Release 25.0 program was used to perform statistical analyses (SPSS Inc, Chicago, IL, USA). Using the histogram, Q-Q graphs, and Shapiro-Wilk test, it was determined whether the data obtained from the study were suitable for normal distribution or not. Qualitative data were expressed as % (n). Normally distributed data are expressed as the Mean ± Standard Deviation (SD), while non-normally distributed data are expressed as the Median (25^{th} and 75^{th} Percentiles). Normally distributed data were analyzed using the t-test on independent samples. The Mann–Whitney U test was applied to examine non-normally distributed data. A p value of less than 0.05 was considered as statistically significant.

4. FINDINGS AND RESULTS

4.1. Clinical findings

Dysuria, anorexia, haematuria and polyuria were found in 64 percent (46/69) of the patient cats in the study. Anorexia, anuria, vomiting, dehydration, and depression were found in 36% (23/69) of the patients.

4.2. Vital parameters findings

There was no statistically significant difference between the groups in terms of temperature, heart rate and respiratory rate (Table 4).

Table 4. Comparison of physical examination parameters between groups.

	Group 1 (Healthy) (n=10)	**Group 2 (Patient) (n=69)**	**p values**
RR (per min)	40.88 ± 4.62	41 .63 ± 8.01	0.918
HR (bpm)	113.00 ± 20.50	104.78 ± 21.01	0.110
T(°C)	38.15 ± 0.32	38.25 ± 1.02	0.201

Data were expressed as mean ± standard deviation. RR: Respiratory rate, HR: Heart rate, T: Temperature. The same capital letters in the same line indicate the similarity between the groups, and different letters indicate the difference. p: Shows the significance of the difference between groups.

4.3. Diseases Diagnosed in Patients.

In the study group, there were 69 sick cats. The diseases diagnosed in the cats were listed in Table 5.

Table 5. The distribution of diseases is presented.

Disease	Frequency	Percentage
Healthy	10	12.7%
ARF	8	10.1%
ARF + LUTD	17	21.5%
CKD	11	13.9%
CKD + LUTD	12	15.18%
HDR	1	1.3%
LUTD	20	25.3%
Total	79	100.0%

Data were expressed as percentage. ARF: Acute renal failure, CKD: Chronic kidney, LUTD: Lower urinary tract diseases disease, HDR: Hydronephrosis.

4.4. Biochemical Findings

The mean BUN, Creatinine and Phosphorus values of the sick cats were found to be significantly higher than those of healthy cats ($P<0.05$).

Table 6. Comparison of biochemical parameters between groups.

	Group 1 (Healthy) (n=10)	Group 2 (Patient) (n=69)	p values
BUN (mg/ml)	15.18 ± 6.58 A	79.58 ± 71,72 B	<0.001**
Cre (mg/ml)	0.80 ± 0.21 A	3.68 ± 4.47 B	<0.001**
P (mg/ml)	5.04 ± 1.68 A	7.24 ± 3.56 B	0.017*

Data were expressed as mean ± standard deviation. The same capital letters in the same line indicate the similarity between the groups, and different letters indicate the difference. * $p<0.05$, ** $p<0.001$. BUN: Blood Ure Nitrogen, P: Phospor. Cre: Creatinine.

4.5. Complete Blood Count Findings

The sick cats' mean lymphocyte levels were significantly lower than that of healthy cats ($P<0.001$). There were no significant differences in other haematological variables between the study and control group.

Table 7. Comparison of complete blood count parameters between groups.

Parameters	Group 1 (Healthy) (n=10)	Group 2 (Patient) (n=69)	p Values
WBC (10^9/L)	8.71 ± 4.30	10.81 ± 7.57	0.906
LYMPH (10^9/L)	4.13 ± 1.87	2.05 ± 3.61	<0.001**
MON (10^9/L)	0.50 ± 0.15	0.65 ± 0.58	0.903
GRAN (10^9/L)	10.46 ± 2.79	10.16 ± 6.22	0.854
RBC (10^{12} /L)	8.50 ± 1.29	7.95 ± 3.20	0.729
HGB (g/dl)	12.35 ± 1.94	12.94 ± 3.84	0.295
HCT (%)	34.69 ± 3.72	33.85 ± 10.39	0.959
MCV (fl)	42.22 ± 4.81	44.45 ± 6.83	0.077
MCH (Pg)	16.11 ± 1.90	19.33 ± 6.60	0.098
MCHC (g/dl)	34.00 ± 2.82	36.55 ± 5.39	0.092
EOS (%)	1.88 ± 0.78	2.95 ± 3.99	0.298

Data were expressed as mean ± standard deviation. The same capital letters in the same line indicate the similarity between the groups. and different letters indicate the difference. * P<0.05. ** P<0.001.

WBC: White blood cells, LYMPH: Lymphocytes, MON: Moncytes, GRAN: Granylocytes, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, EOS: Eosinophil.

4.6. Urine Dip-Stick Examination Findings

The patient cats' mean specific gravity, blood, Ph, protein and WBC values were significantly higher than the references values reported for cats. There were no significant differences between the study and control groups in other variables.

Table 8. Comparison of urine dip-stick parameters between groups (mean ± standard deviation)

Parameters	(Patients)(n=69)	Reference range (Yadav et al., 2020)
Urobilinogen (mg/dl)	0.10 ± 00.00	Negative
Glucose (mg/dl)	8.09 ± 36.25	Negative
Specific Gravity	1039.70 ± 14.60	≥ 1.035
Blood (RBC/µL)	38.08 ± 103.37	Negative
pH	6.49 ± 9 .00	6-7.5
Protein (mg/dl)	149.54 ± 123.17	Undetectable
WBC (WBC /µL)	269.85 ± 232.54	five WBCs may be present per HPF.
Nitrite	Negative	Negative
Bilirubin	Negative	Negative
Ketones (mg/dl)	Negative	Negative

Data were expressed as mean ± standard deviation. HPF= High power field.

4.7. Urine Sediment Examination Findings

Table.9 reports the the percentage of patient cats for each grade of urine sediment examination parameters. According to Kidney Epithelial cells, it was given on the table s that in grade 0, the percentage of patients were 0 %. In grade 1, the percentage of patient cats were (43.5%) and in grade 2, the percentage of patient cats were (56.5%). According to the crystal parameters;the existence of crystal parameter in patient cats were (47.8%) which was distributed as follows: Struvite (%21.8); Amorphous Phosphate (8.7%); Amorphous Urate (8.7%); Calcium Oxalate (4.3%) and Calcium Carbonate (4.3%). With respect to bacteria, grade zero percentage of bacteria in patient cats were (0%). Regarding grade 1; the percentage of bacteria in patient cates were (74%) and with regard tograde 2, it was (26%). Furthermore, the percentage of RBC in the grade zero patient were (34.8%). For grade 1, the percentage of RBC in patient cats were (34.8%). Meanwhile, the percentages for grade 2 were (30.4%). And finally, the percentage of WBC in patient cats for grade zero were (4.3%), for grade 1 were (30.5%) and for grade 2 it was (65.2%).

Table 9. Urine sediment parameters of the patients (urine group)

Urine Sediment results	Grade	(Patient) (n=23)
Kidney Epithelial cells	Grade 0	%0 (0/23)
	Grade 1	%43.5 (10/23)
	Grade 2	%56.5 (13/23)
Crystal	No	%52.2 (12/23)
	Struvite	%21.8 (5/23)
	Amorphorus Phosphate	%8.7 (2/23)
	Amorphorus Urate	%8.7 (2/23)
	Calcium Oxalate	%4.3 (1/23)
	Calcium Carbonate	%4.3 (1/23)
Bacteria	Grade 0	%0 (0/23)
	Grade 1	%74 (17/23)
	Grade 2	%26 (6/23)
RBC	Grade 0	%34.8 (8/23)
	Grade 1	%34.8 (8/23)
	Grade 2	%30.4 (7/23)
WBC	Grade 0	%4.3 (1/69)
	Grade 1	%30.5 (7/23)
	Grade 2	%65.2 (15/23)

Grade 0: No cells, grade 1: (1-5) cells, grade 2: more than six cells in high power field. Data were expressed as % (n/total).

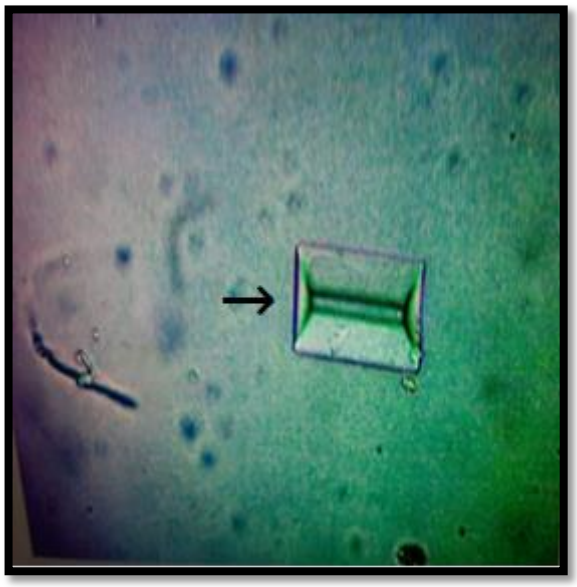


Figure 6. Cat with LUTD revealed Struvite Crystal during examination of urine sediment(40x).

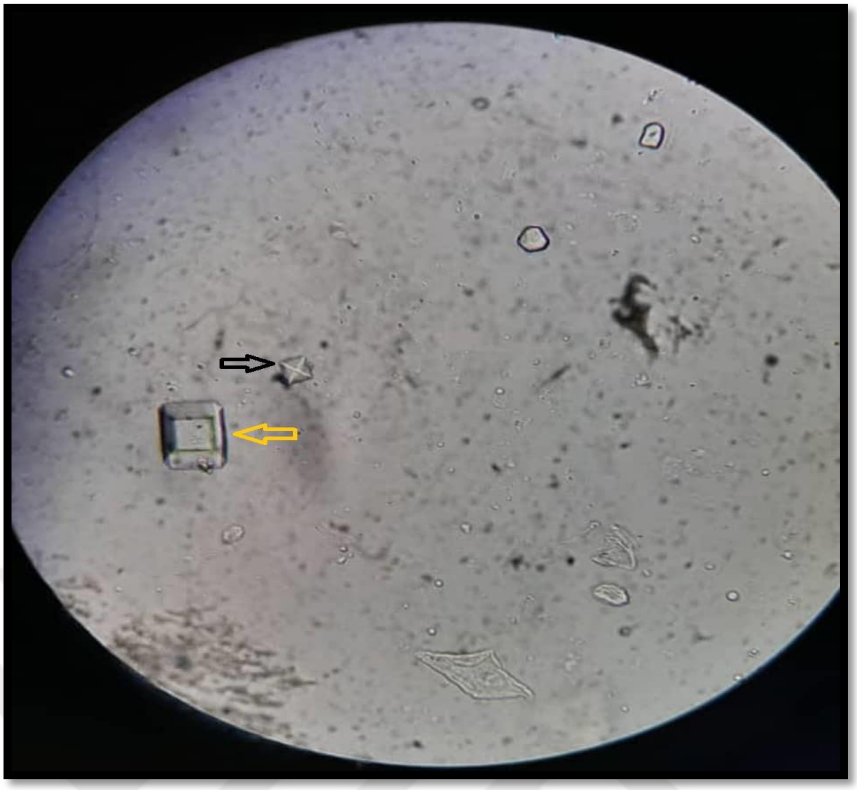


Figure 7. Cat with urolithiasis revealed calcium oxalate crystal (black arrow) and struvite (yellow arrow) in urine sediment examination (40x).

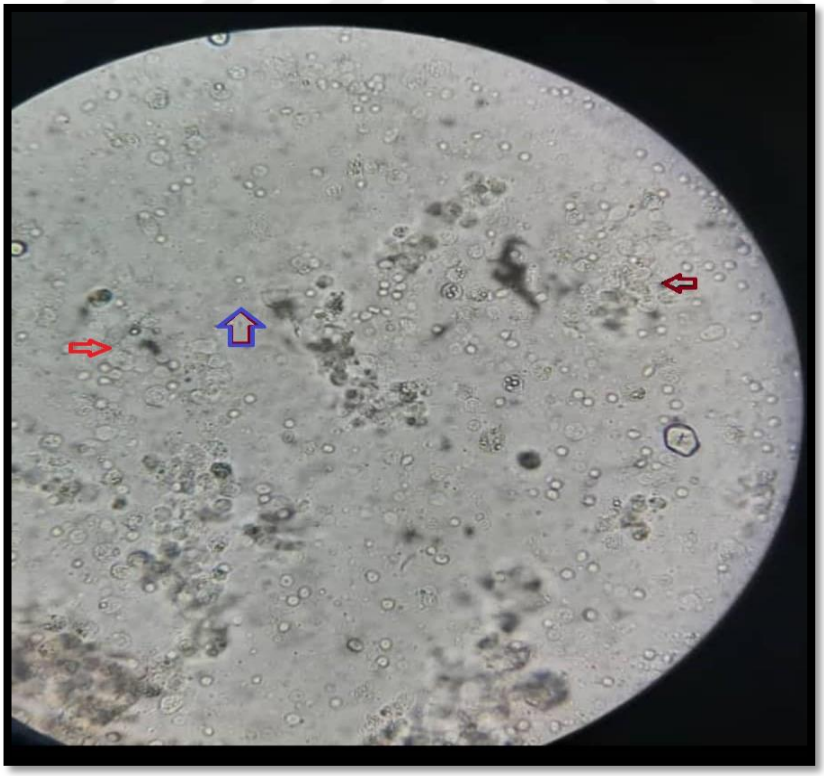


Figure 8. Neoplastic transitional epithelial cell (red arrow) and microorganism (blue arrow) in cat urine sediment (40x) with LUTD.

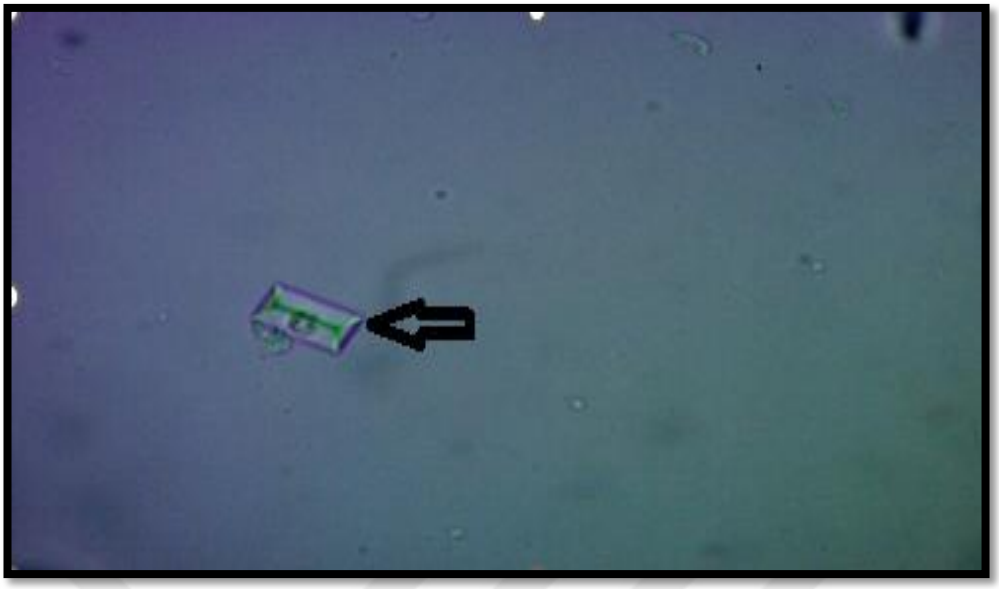


Figure 9. Cats with LUTD showed struvite (black arrow) crystal in urine sediment analysis (40x).

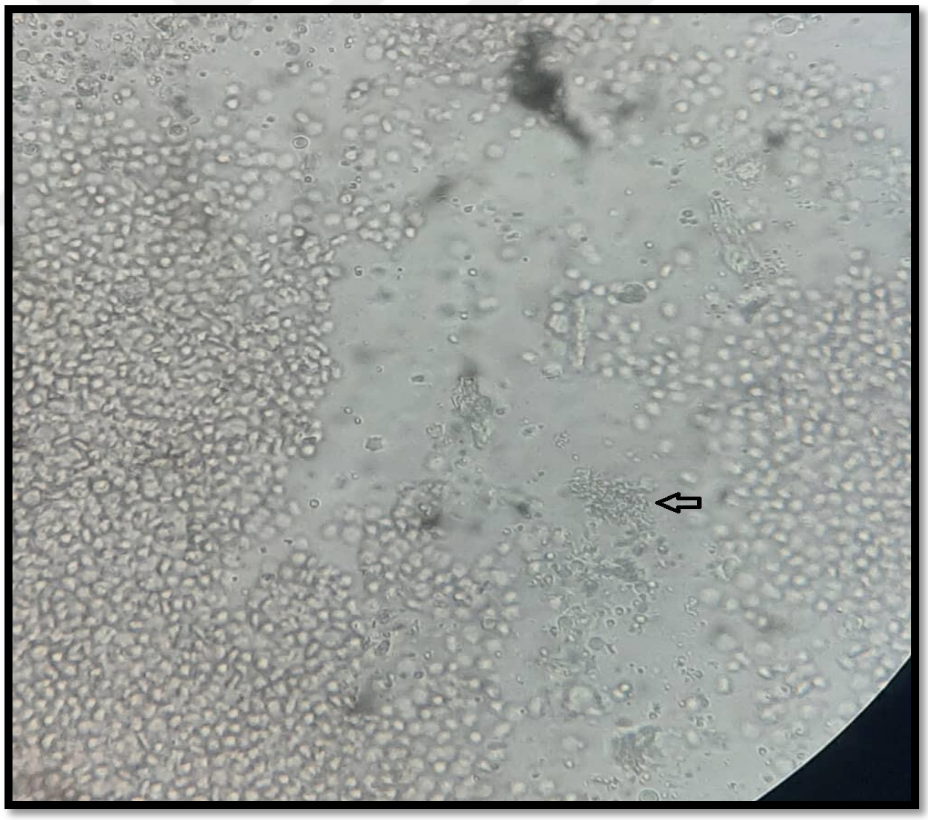


Figure 10. Cats with LUTD showed amorphous (black arrow) crystal in urine sediment analysis (40x).

4.8. Renal USG Findings

The patient cats' average Vesica Urineria Wall Thickness (VUMT) values were greater than the reference values. On the other hand, the patient cats' right kidney cortical thickness (RKCT), right kidney medulla thickness (RKMT), left kidney cortical thickness (LKCT), left kidney medulla thickness (LKMT) values were lower than the reference values.

Table 10. Comparison of renal USG parameters with reference ranges.

Parameters	**Patient** (n=69)	**Reference range** (Park et al., 2008; Debruyn et al., 2012; Maleki, 2011)
VUWT (cm)	0.23 ± 0.93	(0.13-0.17) cm
RKL (cm)	4.01 ± 0.92	(3-4.3) cm
RKW (cm)	2.72 ± 0.31	(3-4.3) cm
RKCT (cm)	0.73 ± 0.25	(0.47-0.08) cm
RKMT (cm)	0.53 ± 0.15	(0.55-0.07) cm
LKL (cm)	3.77 ± 0.74	(3-4.3) cm
LKW (cm)	2.74 ± 0.69	2.76 ± 0.29 2.90 (2.45-3.04)
LKCT (cm)	0.67 ± 0.25	(0.47-0.08) cm
LKMT (cm)	0.50 ± 0.16	(0.55-0.07) cm

Data were expressed as mean ± standard deviation. VUWT: Vesica Urineria Wall Thickness. RKL: Right kidney length. RKW: Right kidney width. RKMT: Right kidney medulla thickness. RKCT: Right kidney cortical thickness. LKL: Left kidney length. LKW: Left kidney width. LKCT: Left kidney cortical thickness. LKMT: Left kidney medulla thickness.

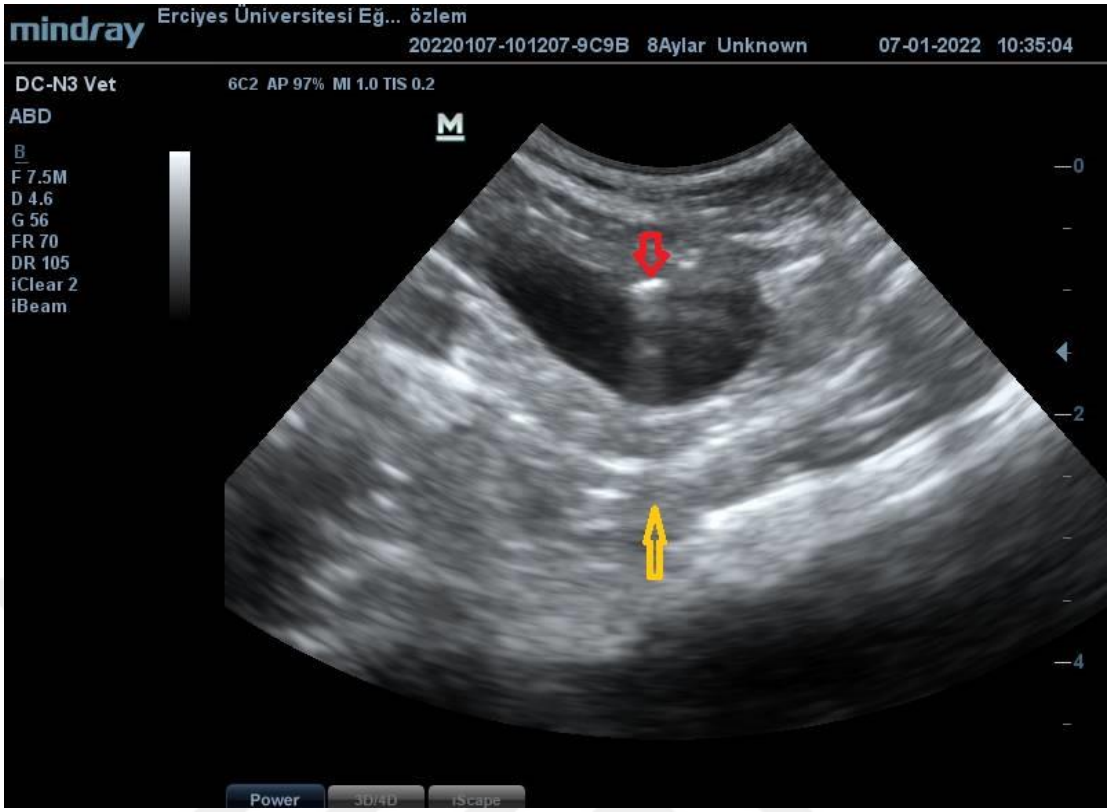


Figure 11. This sonogram (micro-convex transducer, 7.5 MHz) shows presence of urinary bladder stone (red arrow) with shadow (yellow arrow).

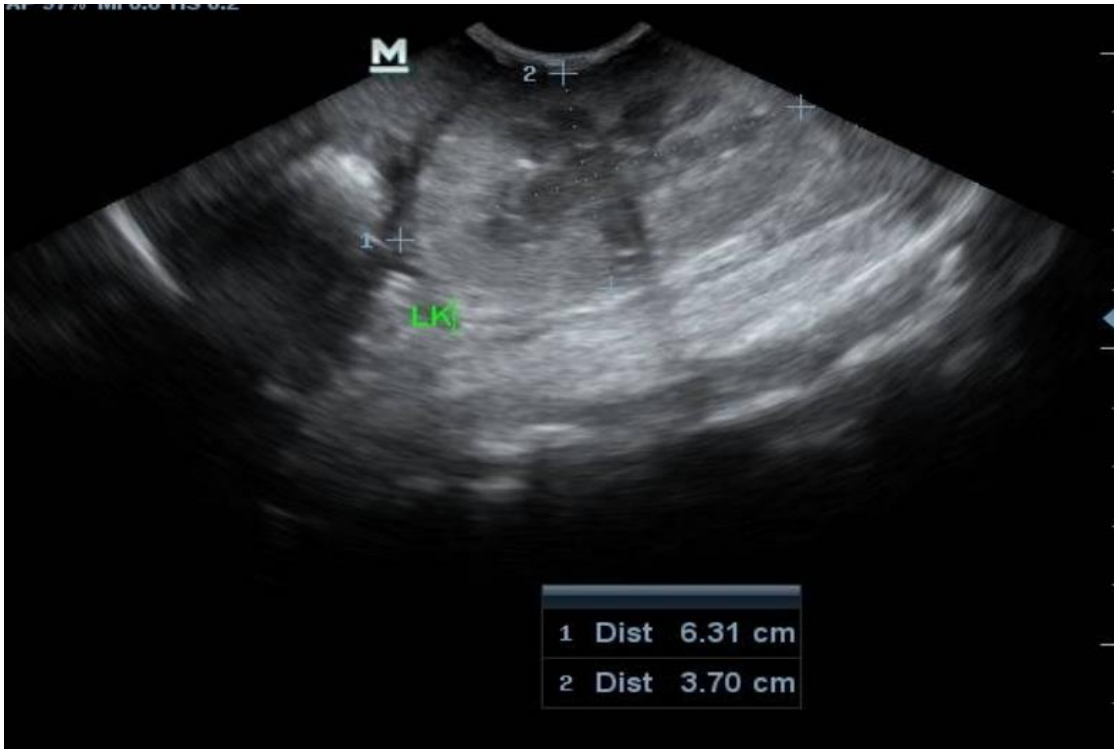


Figure 12. In this sonogram (micro-convex transducer, 7.5 MHz) increased length of the left kidney; 6.31 cm, dilatation renal pelvis, increase echogenicity of the cortex.

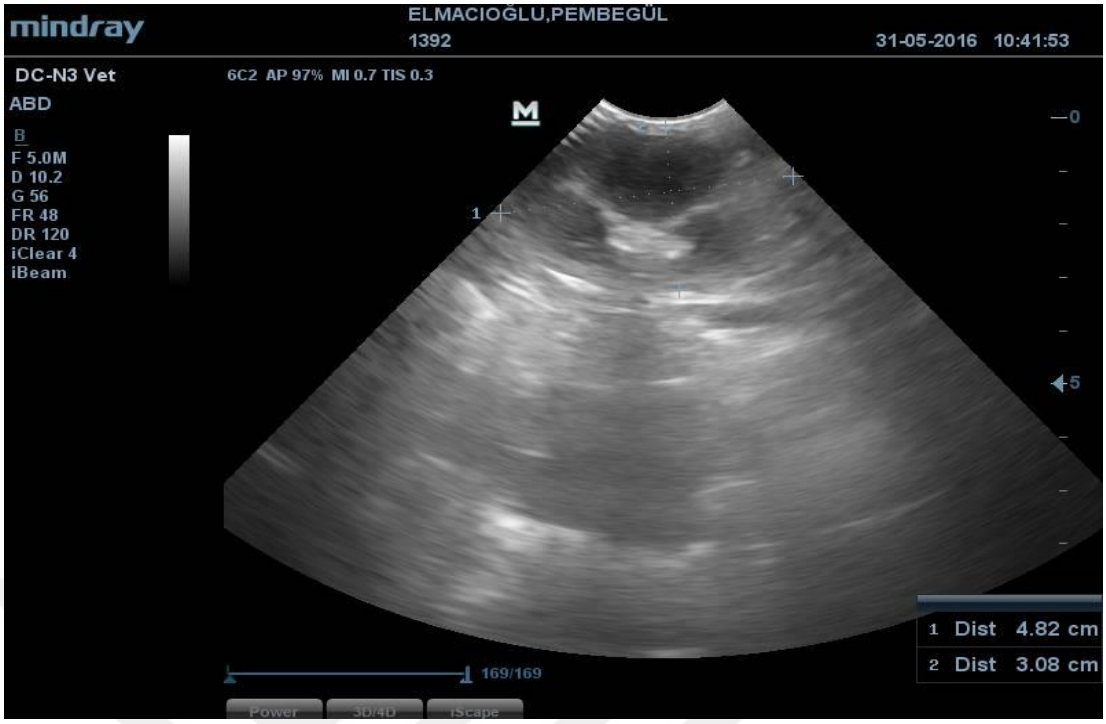


Figure 13. This ultrasonography (micro-convex transducer, 7.5 MHz) revealed an enlarged kidney length of 4.82 cm, dilatation of the renal pelvis, and enhanced echogenicity of the cortex in cat with ARF.

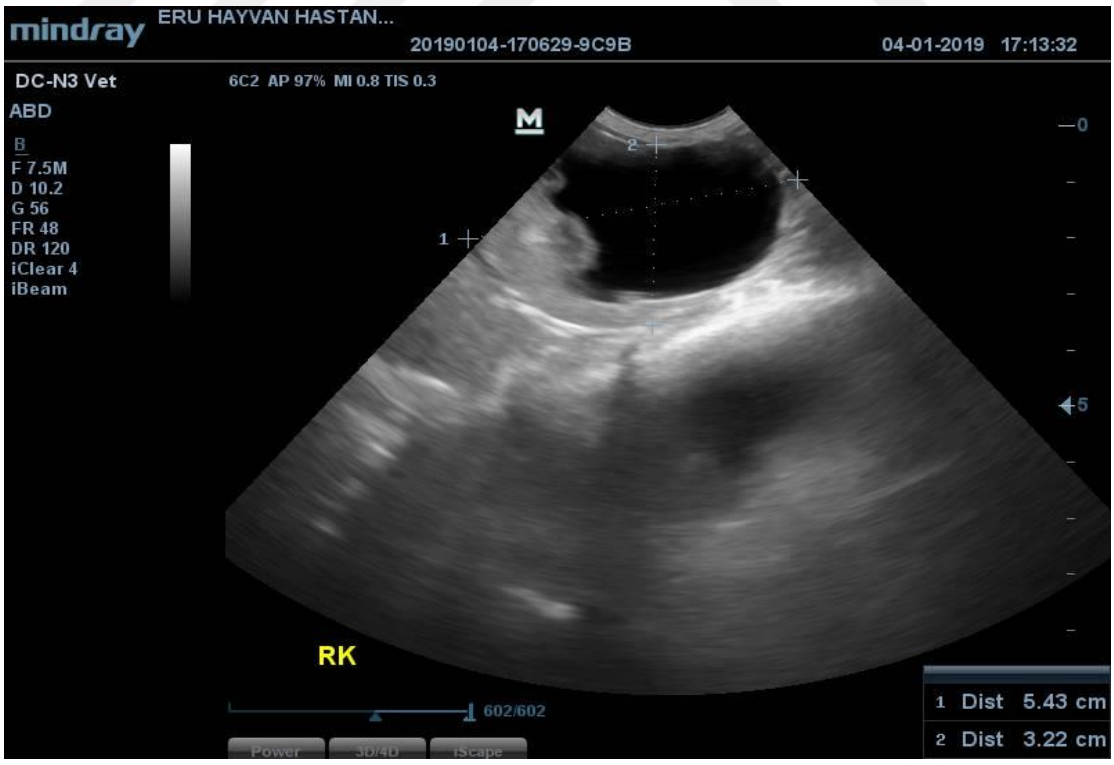


Figure 14. This sonogram (micro-convex transducer, 7.5 MHz) revealed an enlarged length of the right kidney; 5.43 cm, absence of medulla and cortex differentiation, and presence of anechoic region (fluids) in cats with hydronephrosis.

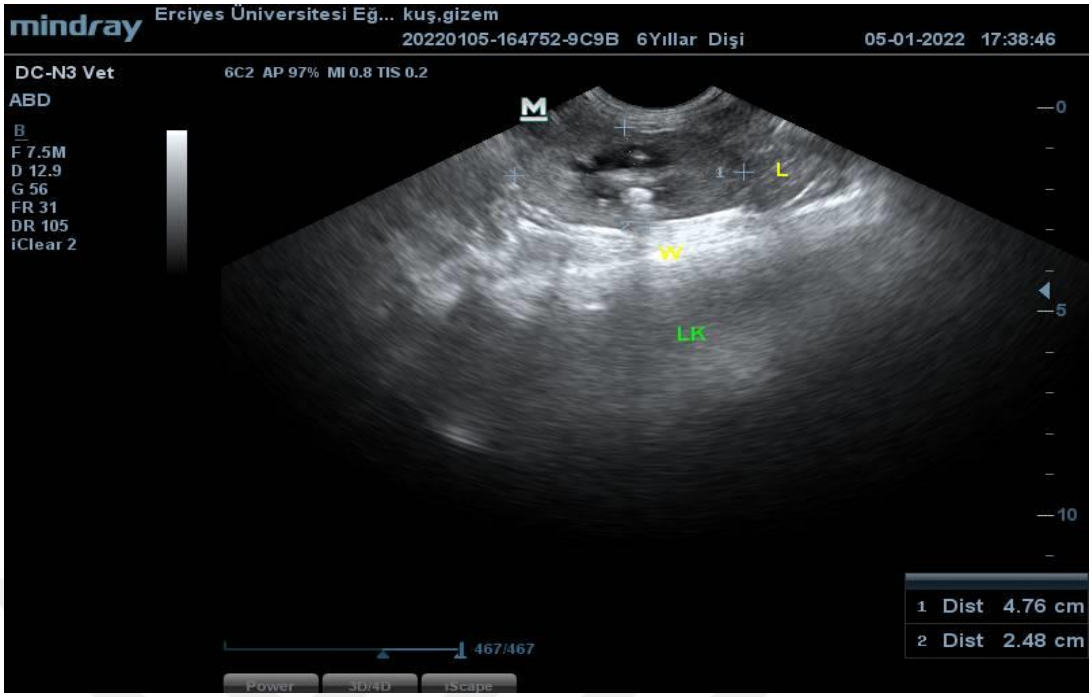


Figure 15. This ultrasonography (micro-convex transducer, 7.5 MHz) revealed an enlarged left kidney length of 4.71 centimetres, dilatation of the renal pelvis, renal calculi with shadow, and increased echogenicity of the cortex.

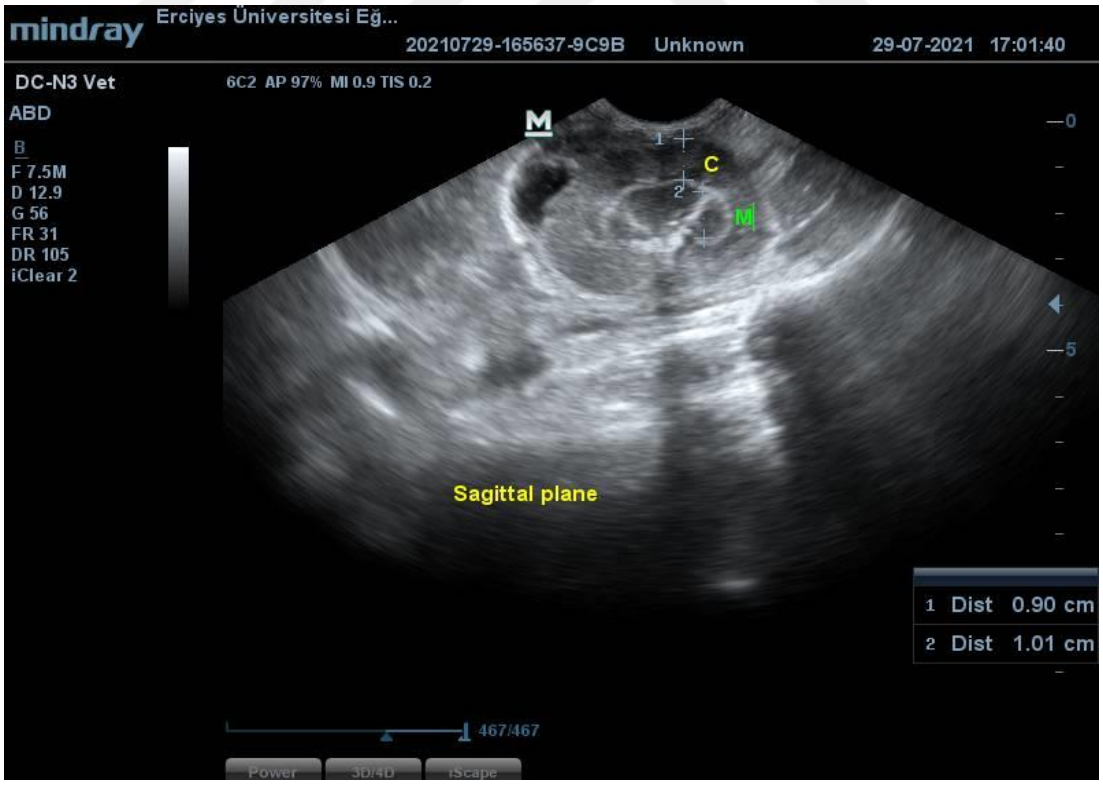


Figure 16. Representative ultrasonographic images from cat (micro-convex transducer, 7.5 MHz) demonstrating the medullary rim sign (MRS) in the renal parenchyma, which can be observed in kidney disorders.

4.9. Clusterin analyses findings

The patient cats' median serum Clusterin concentration was determined to be significantly higher when compared with healthy cats ($p<0.05$). These results were presented in the table 11.

Table 11. Comparison of Clusterin levels in patient serum group samples with Clusterin levels of control group.

	Group 1 (Healthy) (n=10)	**Group 2 (Patient, serum group) (n=46)**	**p Values**
Clusterin (μg/ml)	36.42 (29.08.-48.39)	42.87 (34.51-57.17)	P<0.05

Data were expressed as median (25^{th} and 75^{th} Percentiles). The median quantities of Clusterin in urine were found to be significantly lower than Clusterin levels measured in patient serum and healthy cat serum ($p<0.001$). These findings were given in the Table 12

Table 12. Comparison of Clusterin levels measured in healthy, patient serum and urine groups

	Group 1 (Healthy) (n=10)	**Group 2 (Patient, Serum group) (n=46)**	**Group 3 (Patient, Urine group) (n=23)**	**p Values**
Clusterin (μg/ml)	36.43 (29.08-48.39) A	42.87 (34.51-57.17) B	20.59 (16.83-25.91) C	<0.001**

ABC: Different letters in the same row indicate statistical differences between the groups. Data were expressed as median (25^{th} and 75^{th} Percentiles).

In serum samples the levels of average Clusterin were given in the Table 13. According to these findings, cats with chronic renal failure had significantly higher mean Clusterin levels than cats with acute kidney failure ($p<0.001$).

Table 13. Comparison of Clusterin levels measured in serum of cats with acute and chronic kidney failure.

	ARF (n=7)	CKD (n=10)	p Values
Clusterin (µg/ml)	38.80 ± 15.18 A	84.79 ± 61.87 B	<0.001**

Data were expressed as mean ± standart deviation. ARF: Acute renal failure. CKD: Chronic kidney disease.

The average levels of Clusterin in serum samples are shown in Table 14. Cats with acute renal failure had significantly higher mean/median Clusterin levels than cats with lower urinary tract diseases (P=0.0009).

Table 14. Comparison of Clusterin levels measured in cats' serum with urinary tract diseases and acute kidney failure

	LUTD (n=8)	ARF (n=7)	p Values
Clusterin (µg/ml)	23.24 ± 69.12 A	38.80 ± 15.18 B	0.009*

Data were expressed as mean ± standart deviation. LUTD: Low urinary tract disease. ARF: Acute renal failure. Data were expressed as mean ± standart deviation.

The median concentrations of Clusterin in serum samples are presented in Table 15. Cats with chronic kidney failure had significantly higher mean Clusterin levels than cats with lower urinary tract disease (P<0.001).

Table 15. Comparison of Clusterin mean levels measured in serum of patient cats with lower urinary tract diseases and chronic kidney failure.

	LUTD (n=8)	CKD (n=10)	p Values
Clusterin (µg/ml)	23.24 ± 69.12 A	84.79 ± 61.87 B	<0.001**

Data were expressed as mean ± standart deviation. LUTD: Low urinary tract disease. CKD: Chronic kidney disease.

Table 16 presents the mean concentrations of Clusterin in serum samples. Mean Clusterin levels were significantly higher in cats with mix infection (ARF+LUTD) than in cats with lower urinary tract infections (P<0.001).

Table 16. Clusterin levels were evaluated in serum of patient cats with lower urinary tract diseases and cats with mix infection (acute kidney failure with lower urinary tract diseases)

	LUTD (n=8)	**ARF+LUTD (n=12)**	**p Values**
Clusterin (μg/ml)	23.24 ± 69.12	37.13 ± 10.94	<0.001**

Data were expressed as mean ± standart deviation. LUTD: Low urinary tract disease. ARF+LUTD: Acute renal failure with lower urinary tract disease.

The mean concentrations of Clusterin in serum samples are represented in Table 17. According to these findings, mean Clusterin levels were significantly higher in cats with mix infection (CKD+LUTD) than in cats with just lower urinary tract infections (P<0.001).

Tablo 17. Clusterin levels were evaluated from serum of patient cats with lower urinary tract diseases and cats with mix infection (chronic kidney failure and lower urinary tract diseases).

	LUTD (n=8)	**CKD+LUTD (n=9)**	**p Values**
Clusterin (μg/ml)	23.24 ± 69.12	54.75 ± 22.61	<0.001**

Data were expressed as mean ± standart deviation. LUTD: Low urinary tract disease. CKD+LUTD: chronic kidney disease with lower urinary tract disease

Clusterin and BUN exhibited a positive, strong and statistically significant correlation (P0.001, r=0.756). Furthermore, Clusterin and creatinine showed a positive very strong and statistically significant correlation (P0.001, r=0.829). Clusterin and Phosphorus demonstrated a positive, strong and statistically significant correlation (P=0.001. r=0.744) (Table 20).

Table 18. Correlation matrix for Clusterin level

		Clusterin	**BUN**	**CRE**	**P**
Clusterin (µg/ml)	Pearson Correlation	1	0.756**	0.829**	0.744**
	Sig. (2-tailed)		<0.001	<0.001	<0.001
BUN (mg/ml)	Pearson Correlation	0.756**	1	0.820**	0.725**
	Sig. (2-tailed)	<0.001		<0.001	<0.001
CRE (mg/ml)	Pearson Correlation	0.829**	0.820**	1	0.729**
	Sig. (2-tailed)	<0.001	<0.001		<0.001
P (mg/ml)	Pearson Correlation	0.744**	0.725**	0.729**	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	

**. Correlation is significant at the 0.01 level (2-tailed).

In this study, when the cut-off point for serum Clusterin between CKD and ARF were taken as 3.11, sensitivity, specificity area under the ROC curve (AUC) were calculated as 77.8% and 25% respectively. Sensitivity and specificity, likelihood ratio and AUC values belonging to Clusterin variables were also given in Table 21.

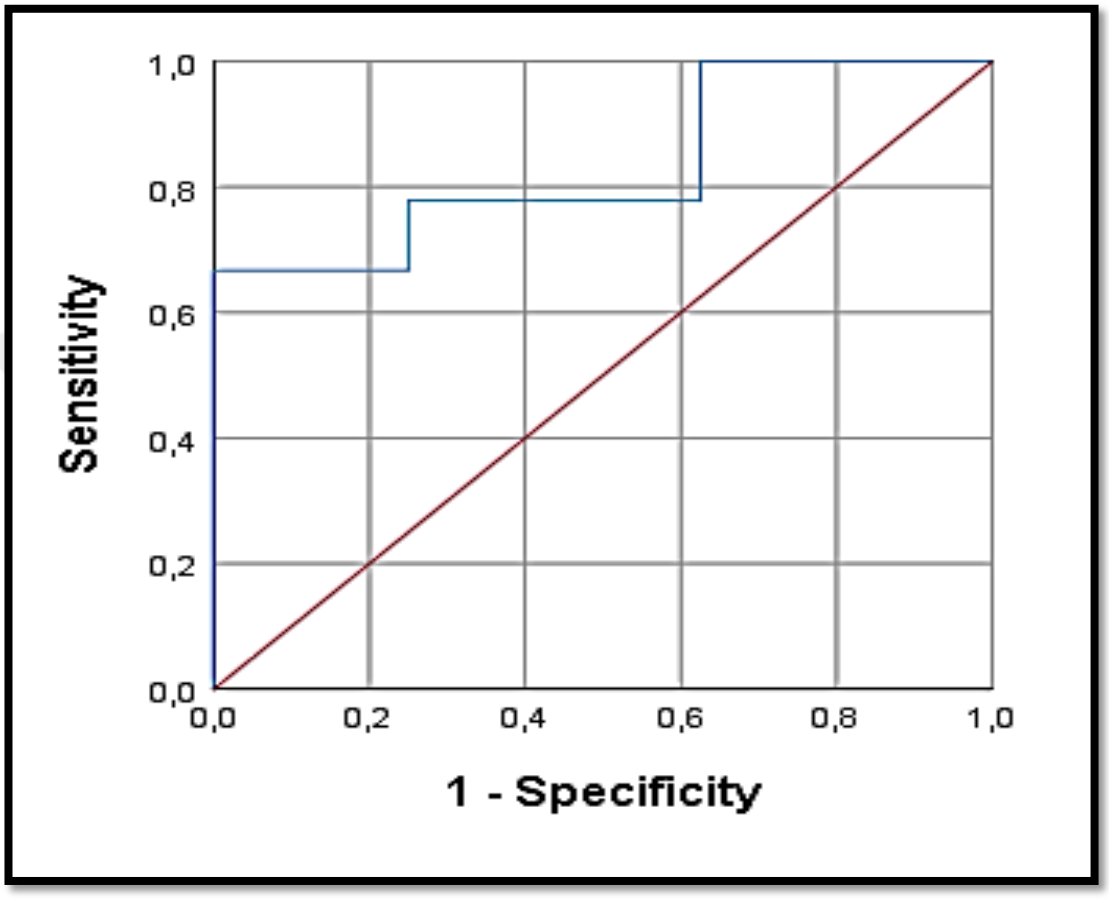


Fig.17 ROC Analysis Of CLUSTERIN In The Serum Of The Patient Cats.

Table 19. Area Under the Curve

AUC	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.833	0.102	0.021	0.633	1.000

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

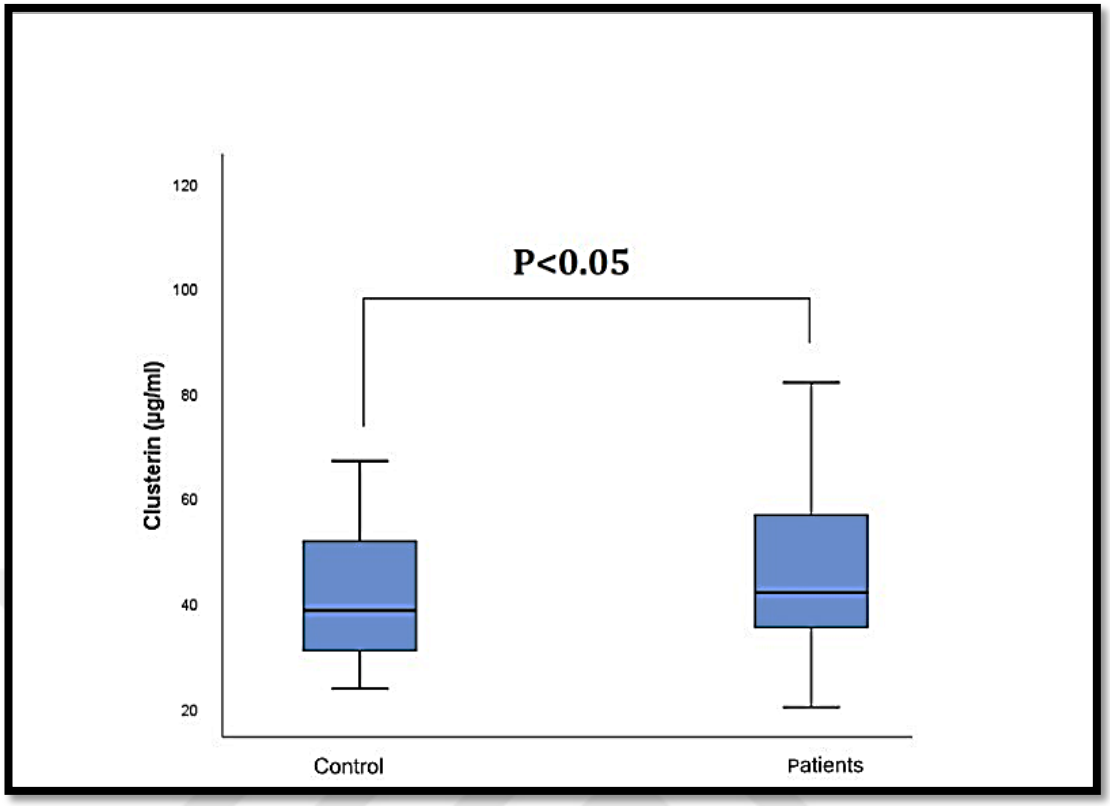


Figure 18. Appearance of the Clusterin levels in patients and control cats using graph.

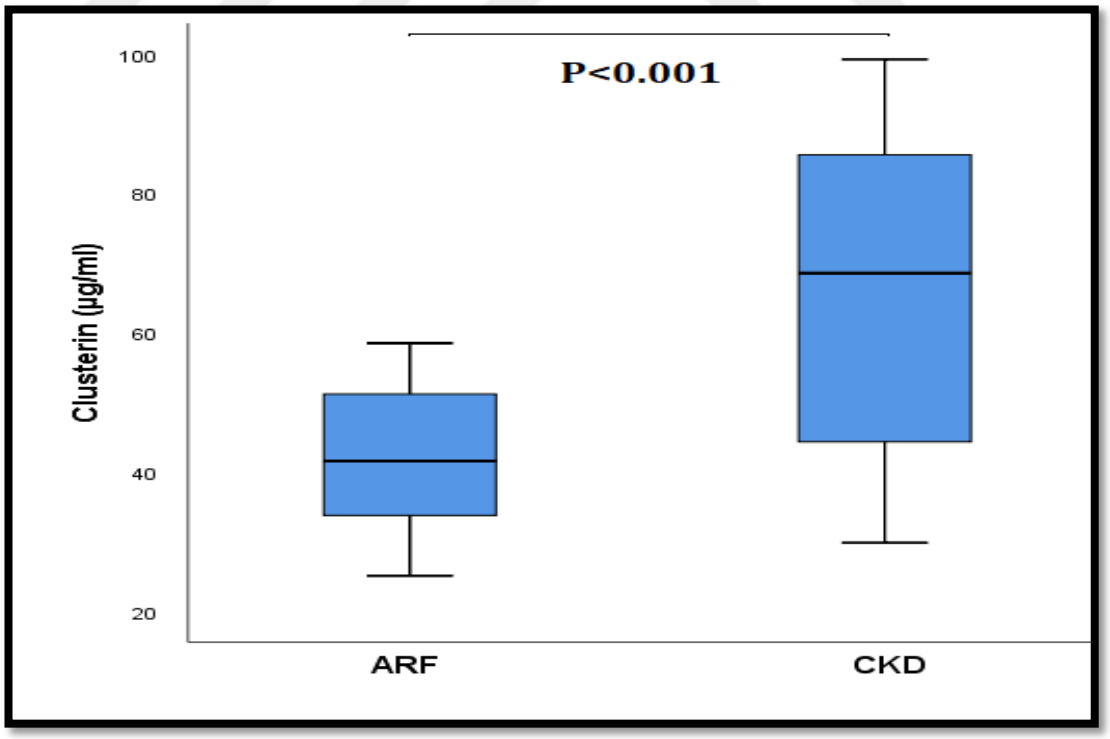


Figure 19. Appearance of the Clusterin levels in cats with ARF and CKD using graph.

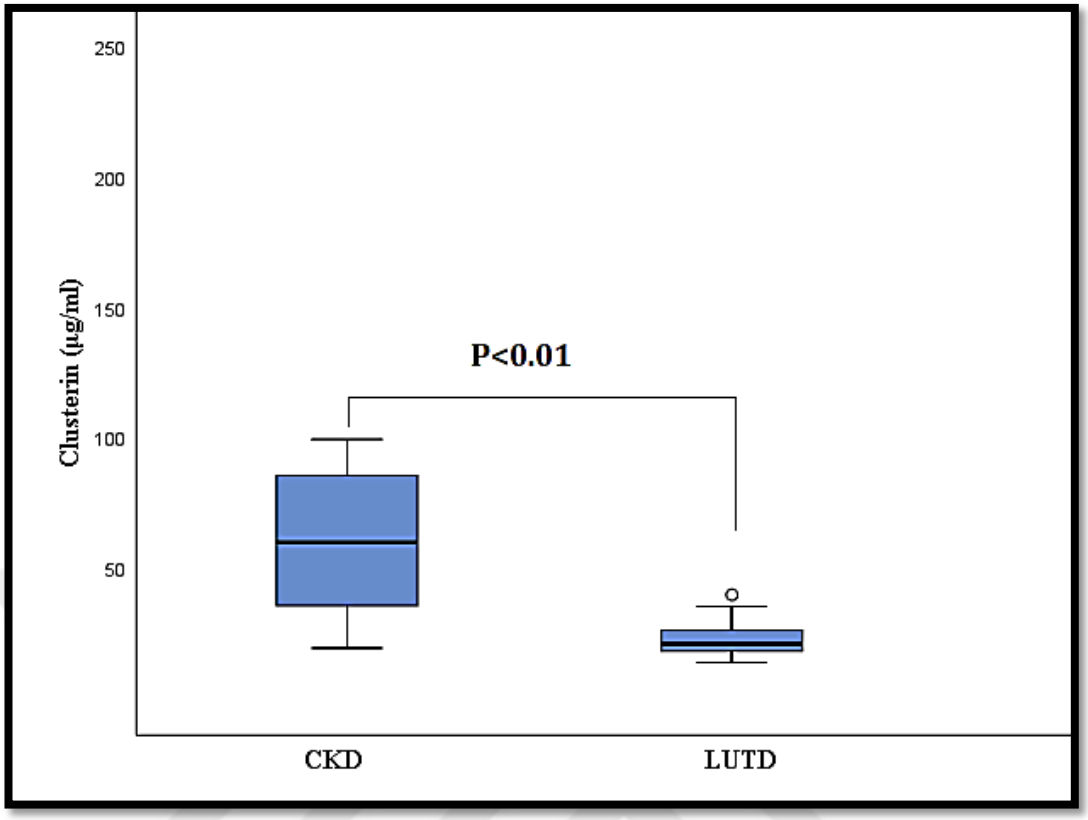


Figure 20. Appearance of the Clusterin levels in cats with LUTD and CKD using graph.

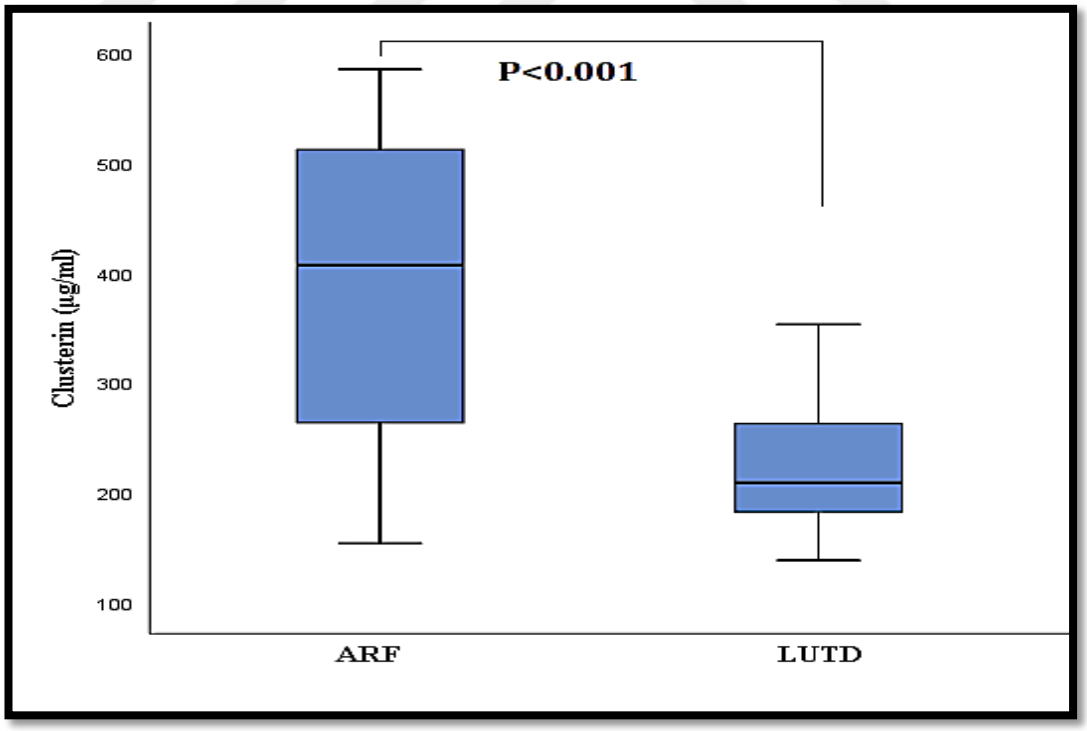


Figure 21. Appearance of the Clusterin levels in cats with LUTD and ARF using graph.

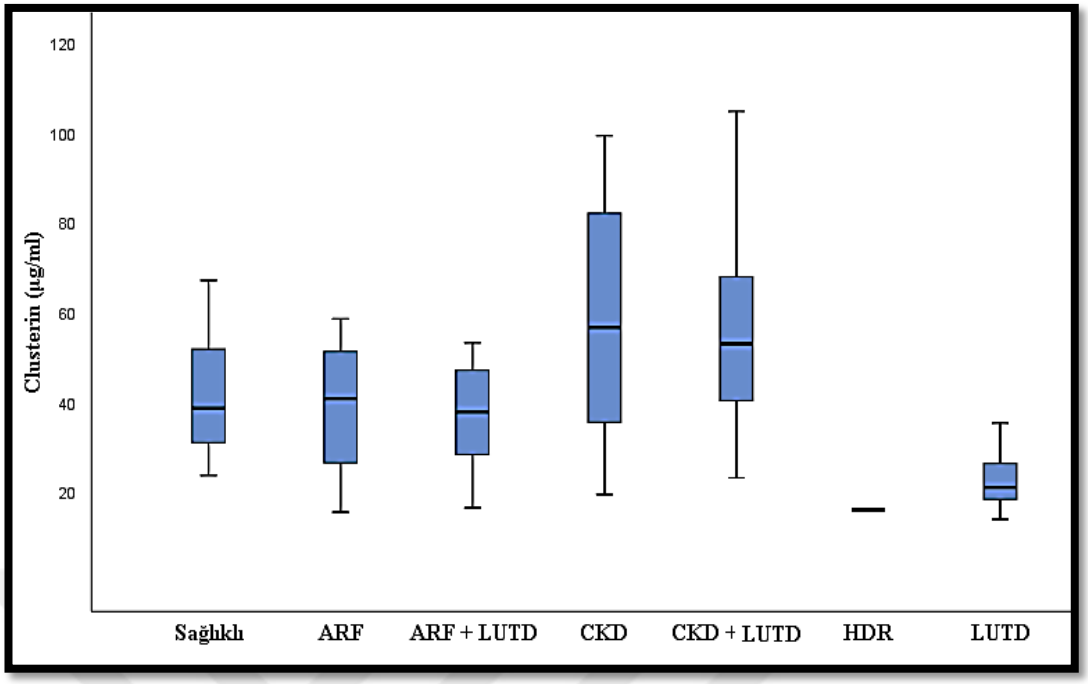


Figure 22. Appearance of the Clusterin levels in patients (ARF, CKD, LUTD, HDR, ARF+LUTD, CKD+LUTD) and control cats using graph.

5. DISCUSSION

Using renal biomarkers to detect renal dysfunction early in cats is crucial, as patients with renal failure have a significant chance of developing permanent kidney damage. Serum concentrations of BUN and creatinine have been used in diagnosing renal failure, but these parameters have also some limitations. Therefore, new studies on renal biomarkers are required. Thus, in this Master thesis, Clusterin levels were investigated to determine whether this biomarker is a valuable renal biomarker for cats or not as Clusterin has recently been studied in dogs and in human medicine for this purpose. Therefore, Clusterin might also be a clinically valuable biomarker in cats.

Acute kidney injury (AKI) has long-term effects that include progressive function loss and the development of chronic kidney disease (CKD). Clusterin (CLU) protein resembling a chaperone are proteins that assist the conformational folding or unfolding of large proteins or macromolecular protein complexes. There are a number of classes of molecular chaperones, all of which function to assist large proteins in proper protein folding during or after synthesis, and after partial denaturation. Chaperones are also involved in the translocation of proteins for proteolysis.Clusterin (CLU) improves tissue healing after ischemia-reperfusion damage (IRI) in the kidney (Guo et al.. 2016). Clusterin is a complex and paradoxical apolipoprotein, has attracted an increasing number of scientists in recent years. This mysterious protein is present in all body fluids besides the intracellular matrix and it performs various and sometimes contradictory roles in an increasing number of diseases. Clusterin appears to alter its location and function to ensure cellular survival as a cytoprotective factor, hence its importance in neuroprotection also in cancer and chemotherapy resistance. However, it can also cause cellular arrest and

apoptotic modulation. In addition, it has been connected to pain modulation, Inflammation, Cardioprotection, satiety and appetite and possibly the development of addictive behavior. Clusterin has been shown to play a significant role in the pathogenesis of a wide variety of diseases including glomerulonephritis, polycystic kidneys, renal tubular diseases, neurodegenerative disorders, Atherosclerosis, myocardial infarction and malignant tumors according to experimental study (Rosenberg and Silkensen, 1995). Consequently, it has been proposed as a biomarker and a highly exploratory novel treatment target for a variety of diseases (Rodríguez-Rivera et al.. 2021).

In the present study; the serum median Clusterin concentration in the healthy cats was determined to be 36.42 µg/ml (29.08.–48.39) while its' concentration in the sick cats was 42.87 µg/ml (34.51–57.17) and in the urine of sick cats was 20.59 µg/ml (16.83–25.59).

In healthy humans and dogs, serum concentrations of Clusterin (60–100 µg/ml) are 1.000 times higher than urinary concentrations (100 ng/ml) (Yerramilli et al.. 2016). Urinary Clusterin concentrations in healthy dogs reported to be 38.50 ng/ml. García-Martínez et al. (2012), used a leishmaniasis model of kidney disease to test U-clusterin as a biomarker of renal impairment in dogs with varying stages. Groups with various stages of renal failure, The dogs with leishmanias were divided into 5 groups: I (n = 9; serum creatinine [SCr] <1,4 mg/dl, urinary protein-to-creatinine [UPC] ratio ≤ 0.5); II (n = 29; SCr <1,4 mg/dl, UPC> 0.5); III (n = 6; SCr ≥ 1,4 mg/dl to <2 mg/dl, UPC> 0.5); IV (n = 13; SCr ≥ 2 mg/dl to <5 mg/dl, UPC> 0.5); and V (n = 7; SCr ≥ 5 mg/dl, UPC> 0.5). Group II urinary clusterin level was (703.6 ng/ml), group III was (2.336 ng/ml), group IV was (4.750 ng/ml), and group V was (4.056 ng/ml).

In the present study; serum Clusterin mean concentrations were determined to be (38.80 ± 15.18) µg/ml in cats with ARF and (84.79 ± 61.87) µg/ml in cats with CKD. Serum Clusterin mean concentration in cats with LUTD included in the study was 23.24 ± 69.12 µg/ml. Serum Clusterin mean concentration in cats with ARF+LUTD was 37.13 ± 10.94 µg/ml and finally serum Clusterin mean concentration in cats with CKD+LUTD was 54.75 ± 22.61 µg/ml.

In the present study, urine Clusterin concentration in only one cat with ARF was included and was found to be 15.41 µg/ml. Urine concentration of Clusterin in only one cat with CKD was 19.38 µg/ml. Urine mean Clusterin concentration in cats with ARF+LUTD (n=5) was 25.70 µg/ml. Urine mean Clusterin concentration in cats with CKD + LUTD (n=3) was 32.34 µg/ml. Finally, urine mean Clusterin concentration in cats with LUTD (n=11) was 19.63 µg/ml.

In the present study, the most crystals that have been found in the urine sediment examinations of patients (n = 23) were struvite (21.8%), amorphous phosphate (8.7%), amorphous urate (8.7%), (4.8%) calcium carbonate and calcium oxalate (4.8%).

In the present study, the median serum Clusterin levels of the cats in the patient group were statistically higher than those of the cats in the healthy group ($p<0.05$). Similarly, García-Martínez et al. (2012) reported, higher urinary Clusterin concentrations in dogs with kidney damage. Experiments have shown that kidney damage or toxic injury lead to increased levels of Clusterin in the urine (Hidaka et al., 2002, Ishii et al., 2007). When the damage occurs in the kidneys tubules and interstitial spaces lead to increase Clusterin levels which occur due to inflammation-associated immune complex deposition and by decreased perfusion via peritubular capillaries which damage tubules and interstitial spaces. An increase in Clusterin production during an ischemia-reperfusion-induced damage is another probable reason (Rosenberg ve Paller, 1991). Renal cell apoptosis, fibrosis, inflammation, ET-1 release, vascular damage. and cellular senescence are all examples of the effects of increased oxidative stress. This can result in an increase in Clusterin synthesis (Spencer et al., 2021).

Cystic epithelial cells expressing Clusterin have been found in a number of human renal diseases, including autosomal dominant and recessive polycystic kidney disease, multilocular cyst of the kidney, cystic renal dysplasia and neoplastic cysts in some cases of Wilms' tumor and renal cell carcinoma (Dvergsten et al. 1994).

.The Clusterin levels in urine were statistically significantly lower than the values measured in serum samples for both patient serum group (n=46) and control group (n=10).The median quantities of Clusterin in urine were found to be significantly

lower than Clusterin levels measured in patient serum and healthy cat serum ($P<0.001$). This finding reduces the diagnostic value of urine Clusterin analysis. Many patients with renal disorders showed reduced urine Clusterin which was considered to lead to renal impairment due to Clusterin deficiency in the urine as reported in the present thesis. For example, Clusterin deficiency, activates the complement cascade which may result in renal injury (Ghiggeri et al., 2002 ; Saunders et al., 1994).

The potential usefulness of serum Clusterin in the diagnosis of renal diseases have been reported several years ago; many patients with renal disorders showed reduced urine Clusterin which was considered to lead to renal impairment due to Clusterin deficiency in the urine as reported in the present thesis. It's interesting to note that renal tubular mesangial and epithelial cells both express Clusterin. Its reduced expression due to glomerulopathy-related proteinuria and worsen postischemic renal damage. After ischemia-reperfuson damage, Clusterin deficient mice reported to develop renal fibrosis and inflammation because of the Clusterin deficiency which act as tissue protector (Guo et al., 2016). In the current study. A urine-specific-Clusterin enzyme immunoassay was not used to detect clusterin levels in urine. Consequently, extremely low concentrations of Clusterin were found in the urine.

In contrast to our findings, It has been reported that animals with kidney damage have a higher concentration of Clusterin in their urine (García-Martínez et al., 2012;Vinken et al., 2012). In addition, experiments have shown that kidney damage or toxic injury lead to increased levels of Clusterin in the urine together with other proteins (Hidaka et al., 2002; Ishii et al., 2007) . According to Wu et al. (2018), Clusterin was considerably higher in the urine of patients with tubulointerstitial renal lesions indicator of tubulointerstitial renal lesions.

Urine Clusterin is a recently found biomarker that correlates with and best predicts ESKD. Incomplete knowledge exists regarding the role of urine Clusterin in the development of acute and chronic renal diseases. Increased Clusterin gene expression in obstructed rabbit kidneys were linked to a reduction in renal concentrating ability and GFR values (Schlegel et al.,1992). Dieterle et al. (2010), compared urine Clusterin with serum blood urea nitrogen (BUN) and creatinine and found a higher diagnostic performance for detecting proximal tubular injury.

To our knowledge this study is the first report on serum Clusterin levels in cats with ARF and CKD. Although, the number of cats with ARF were low cats with CKD had a statistically greater median concentration of serum Clusterin than ARF cats (P=0.021). Clusterin was reported to be involved in a number of distinct mechanisms in response to kidney damage. According to the literature, Clusterin expression is linked to the TGFβ signaling pathway. The progression of fibrosis is accelerated in chronic renal failure. The key fibrosis factor, transforming growth factor beta (TGF-β) plays a significant function. Through activation of the AP-1 transcriptor protein and protein kinase C, TGF-β1 increases clusterin expression in fibrosis,resulting in Clusterin overexpression (Khan and Pandey, 2014).

Increased oxidative stress results in apoptosis, fibrosis and inflammation. ET-1 release vascular damage and cellular senescence in renal cells as similarly observed in our study, this can result in an increase in clusterin synthesis (Spencer et al., 2021). Multiple attacks to the kidney can lead to tubular injury as proposed by the "fibrosis hypothesis" which then triggers inflammation and additional damage to the tubulointerstium, eventually leading to chronic kidney disease. Hodgkins and Schnaper. (2012) reported that, Clusterin association with CKD stage 3 or the development of albuminuria may lend credence to the theory that tubular damage becomes more apparent at this stage.

In ARF, increased oxidative stress can lead to a variety of adverse outcomes, including renal cell death, fibrosis and inflammation. Consequently, this may lead to enhanced Clusterin synthesis. Podocyte expression of Clusterin was confirmed by its co-localization with the podocyte marker WT1 and its upregulation in the glomerulus of both diabetic nephropathy (DN) patients and streptozotocin-induced diabetic mice. Glomerular Clusterin expression is elevated in DN and this may serve to protect podocytes from the apoptosis induced on by oxidative stress (Spencer et al., 2021; He et al., 2020). Accordingly, we might presume that podocytes are more vulnerable to apoptosis brought on by oxidative stress in conditions where kidney damage has occurred, such as in CKD and ARF, and that this leads to an increase in the synthesis and expression of Clusterin.

Mean Clusterin levels were found to be higher significantly (P<0.001) in cats with chronic renal failure compared to cats with lower urinary tract diseases (cystitis, urolithiasis,Flutd, etc.) in the current study. Clusterin has multiple roles in kidney injury. Expression of Clusterin is associated with TGF-b signaling. Chronic renal failure speeds up fibrosis development. TGF- is the principal fibrosis factor. TGF-b1 stimulates the production of Clusterin in fibrosis via activating AP-1 and protein kinase C. Increased oxidative stress leads to the loss of renal cells. The conditions of fibrosis and inflammation, ET-1 release, vascular injury, and cellular senescence can result in a Clusterin extra production (Spencer et al., 2021; Khan and Pandey, 2014). As a result, we can assume that the fibrosis that typically happens in CKD causes an increase in Clusterin production and levels.

In the present study, Clusterin levels were found to be higher significantly (P<0.001) in cats with a mixture of acute renal failure and lower urinary tract diseases than in cats with lower urinary tract diseases alone. Ischemia-reperfusion may increase Clusterin production in ARF. Clusterin protein is synthesized in the renal tubular epithelium in response to the de novo synthesis of Clusterin mRNA in reaction to renal damage (Khan and Pandey, 2014; Rosenberg and Paller. 1991). As a result, we can expecting Ischemia-reperfusion to enhance Clusterin production in ARF. Also, we can hypothesize that podocytes are more prone to apoptosis caused by oxidative stress in conditions characterized by renal damage, such as CKD and ARF, and that this leads to an increase in Clusterin synthesis and expression.

Median Clusterin levels in cats with CKD+LUTD were significantly higher (P<0.001) than those of cats with LUTD alone . There is some evidence that RAS, may influence renal Clusterin expression in response to renal damage. Jung et al. (2014) . Evidence from a nephrectomized rat model showed that damage related increases in Clusterin mRNA and proteinuria were inhibited by an angiotensin-converting enzyme (ACE) inhibitor (Chung et al.,2019). These results imply that the renin-angiotensin system regulates the induction of Clusterin in response to renal damage. Therefore in CKD, clusterin is induced by activation of the RAAS system in response to renal injury, resulting in increased levels of Clusterin. Additionally, we can speculate that in diseases like CKD and ARF, which are defined by renal impairment, podocytes are more vulnerable to apoptosis brought on by oxidative

stress, and that this results in an increase in Clusterin synthesis and expression which can also lead to increase Clusterin levels in CKD and ARF.

In the present study, the median values of BUN,creatinine and phosphorus were significantly higher in sick cats than in healthy cats (P<0.001). According to Finco and Cooper(2000), creatinine levels rise when GFR falls by around 75%. Consequently, a reduction in GFR will lead to a rise in BUN and creatinine levels. In CKD, the kidneys are unable to eliminate phosphorus, resulting in an excess of phosphorus in the blood.

In the present study; Clusterin and BUN exhibited a positive. strong and statistically significant correlation (P0.001. r=0.756). Furthermore. Clusterin and creatinine showed a positive. very strong and statistically significant correlation (P0.001. r=0.829). Furthermore, Clusterin and Phosphorus demonstrated a positive. strong and statistically significant correlation (P=0.001. r=0.744). García-Martínez et al. (2012), demonstrated a 0.86 Spearman correlation between the UCL/Cr ratio and the UPC ratio. suggesting a strong connection between them. Clusterin levels in the urine were discovered to be correlated with total protein (r = 0.28 p = 0.018) and total protein/creatinine (r = 0.26. p = 0.02) levels (Solichova et al.,2007). Clusterin amount in urine was not correlated with glomerular filtration rate, Age and urine GMT/creatinine 1-microglobulin, urine albümin, or urine albumin/creatinine ratio.

The findings observed in the present study showed that the patient cats (urine group) mean specific gravity, blood, Ph, Protein, and WBC values wer significantly higher than the reference ranges. Proteinuria contributes to progressive renal injury as it induces tubular and mesangial damage and toxicity as well as an inflammatory response (Chakrabarti et al.. 2012). Due to LUTD, dipstick parameters such as bacteriuria, pyuria and haematuria also reported to increased (Dorsch et al., 2019).

The feline kidney is frequently imaged with B-mode ultrasonography (Debruyn et al., 2012). It enables superior visualization of the size, shape, and internal architecture of the kidneys (Debruyn et al., 2012). It is possible to restrict the diseased cats manually. According to our findings from the study, the patient cats VUMT values were greater than the reference ranges. Similarly, these values reported to increase due to LUTDs like cystitis and urethral obstruction (Nevins et

al., 2015). On the conversely, the patients cats (RKCT, RKMT, LKCT and LKMT) values were lower than the reference ranges decreases in these parameters. Similarly to our findings, cortical thickness renal cortical thickness reported to reduc in cats with renal function loss (Yan et al., 2020). In the later stages of chronic kidney disease (CKD), there is a reduction in renal size and shape abnormalities associated with the loss of corticomedullary differentiation (Yan et al., 2020).

Limitations and prospects for future research

- First of all, the main limitations of our study is that; cat clusterin-specific Elisa kits were not available because its production completely stopped. Instead, we used dog spesific Clusterin Elisa kit. We did not use a specific Clusterin ELISA kit for measuring urinary level, but we did use a specific kit for measuring Clusterin serum levels.
- The future research might also extend the sample size for more reliable results.
- It is necessary to conduct histopathological examinations to compare the histopathology results and Clusterin levels in ARF, CKD and LUTDS.

6. CONCLUSION AND SUGGESTIONS

Most urine sediment crystals determined in our study was struvite, amorphous phosphorus, amorphous urate and calcium carbonate. The concentration of Clusterin in the serum of sick cats was greater than that of healthy cats, whereas the concentration of Clusterin in the urine of sick cats was significantly lower than that of healthy serum. Serum Clusterin levels were twice as high in serum of cats with CKD compared to cats with ARF. Cats with ARF had higher serum concentrations of Clusterin than cats with LUTD. Serum Clusterin concentrations in cats with CKD were three times higher than in cats with LUTDs. Cats with ARF+LUTDs had higher serum concentrations of Clusterin than cats with LUTDs alone. Serum concentrations of Clusterin in cats with CKD+LUTDs were twice as high as in cats with LUTDs alone.

In addition, the data obtained in the present research could serve as bases for the relevant research area. The results of the present study indicate that the competitive enzyme immunoassay for dogs can be used effectively to measure the concentration of feline Clusterin levels in the serum of cats with ARF and CKD. Clusterin seems effective biomarker in the assessment of CKD and ARF in cat serum.

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RESUME

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