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Research Article

Investigation of the Presence of Crystalluria in Cats with Lower Urinary Tract Disease

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Abstract

Crystalluria, the presence of crystals in urine, results from electrolyte and/or substance oversaturation. Feline lower urinary tract disease (FLUTD), where crystalluria is an etiological factor, is diagnosed by exclusion due to its multifactorial nature and overlapping clinical signs. Urinalysis and sediment examination are practical, cost-effective diagnostic tools. This study investigated crystalluria in domestic cats with FLUTD, classified crystal types, and assessed their role in preventing urethral plug formation and obstruction. It included 50 owned cats (28 males, 22 females, median age: 3 years, range: 1–8) presented to the Harran University Veterinary Faculty Animal Hospital between March 2024 and January 2025. All underwent physical, laboratory, and imaging exams and were classified into four crystalluria subgroups: calcium oxalate (CaOx, n = 9), hippuric acid (n = 8), uric acid (n = 8), and struvite (n = 25). Crystalluria was more frequent in tabby cats fed commercial dry food than in those on home-prepared diets. It was also more common in semi-indoor, neutered cats, particularly those that had never mated. The predominant clinical signs were stranguria and hematuria. Median urine pH was 6.75 (range: 4.0–8.5), and urine specific gravity (Sg) was 1.030 (range: 1.030–1.040). In conclusion, the most prevalent crystals in Şanlıurfa cats were struvite, CaOx, hippuric acid, and uric acid. Crystalluria was most common in male, neutered, tabby cats aged ≥ 3 years, weighing ~ 3.5 kg, fed commercial dry food, never mated, and living a semi-indoor lifestyle. Regular urinalysis and veterinary check-ups are advised for at-risk cats to prevent complications like urethral plug formation and obstruction.

Keywords: Cat, cystitis, urinalysis, ultrasound

Introduction

Feline lower urinary tract disease (FLUTD) is characterized by clinical signs such as stranguria, pollakiuria, hematuria, and periuria (Black, 2018). In the United States, an estimated 250,000–500,000 cats are affected annually (Kalkstein et al., 1999). Studies indicate that feline idiopathic cystitis (FIC) accounts for 55–60% of FLUTD cases, followed by urolithiasis (12–22%) (Saevik et al., 2011). Bacterial urinary tract infections are reported in 1.5–20% of cases, while bladder wall neoplasms and neurological disorders are less common, occurring in 0.3–3.6% and 0.2–3% of cases, respectively (Kaul et al., 2019). When no causative agent is identified, the diagnosis of FIC is made if submucosal petechial hemorrhages are observed via cystoscopy or vesicostomy (Westropp and Buffington, 2004). However, in half of non-obstructive FLUTD cases, the underlying cause remains unknown (Forrester and Towell, 2015).

Crystalluria is the presence of crystals in urine, typically resulting from the oversaturation of certain electrolytes and substances. Key contributors include calcium oxalates, urates, and phosphate ions excreted by the kidneys, which exceed the urine's ability to dissolve and retain them (Dorsch et al., 2014). Crystals may become trapped in the matrix, forming a plug. Severe crystalluria can lead to urethral obstruction even without a colloid matrix, though it is often subclinical. However, persistent or severe crystalluria may predispose to urolith formation, increasing the risk of urethral obstruction and cystitis (Kruger et al. 2003; Dorsch et al., 2014).

The diagnosis, treatment, and management of FLUTD-related conditions such as FIC, crystalluria, and bacterial cystitis face two major challenges: the unidentified cause and the lack of randomized controlled trials evaluating treatment options. Similar difficulties exist in human interstitial cystitis. Due to the multifactorial nature of FLUTD and overlapping clinical signs, diagnosis is often by exclusion. Urinalysis and sediment examination are practical, cost-effective tools providing valuable clinical insights. Since the diagnosis of FLUTD is one of exclusion, it often necessitates various, and sometimes costly, diagnostic tests. Therefore, evaluating the demographic characteristics of cats in the region and assessing the diagnostic utility of

routine clinical parameters may help streamline and facilitate the diagnostic process. This study aims to investigate crystalluria in owned cats with FLUTD, classify crystal types, and assess their role in complications such as urethral plug formation and obstruction. Determining the regional prevalence of crystalluria may help mitigate FLUTD risk and guide pet owners in preventive care.

Material and Method

This study received approval from the Local Ethics Committee for Animal Experiments at Harran University on 04/07/2024 with session 2024/004 and decision number 01-18. All animal owners gave their consent for participation in the study.

Animal Material

This study included 50 owned cats of both sexes presenting with FLUTD-related clinical signs, such as stranguria, pollakiuria, hematuria, and periuria. These cats were admitted to the Harran University Faculty of Veterinary Medicine Animal Hospital for diagnosis and treatment between March 2024 and January 2025. Detailed anamnesis, demographic data, urine dipstick and sediment analysis, and urinary bladder ultrasonographic findings were evaluated.

Clinical Examinations

All cats included in the study underwent a detailed medical history review, demographic data collection, and a comprehensive physical examination. The examination included body temperature measurement, capillary refill time assessment, body weight recording, heart and respiratory rate evaluation, lung and heart auscultation, as well as palpable lymph node and mucosal assessments. Information was recorded on diet, breed, lifestyle (indoor or semi-indoor), neuter status, previous mating history, and whether the cats were adopted or homebred. Additionally, the duration of FLUTD symptoms before hospital admission was documented.

Inclusion/Exclusion Criteria

Cats with a history of any disease other than FLUTD, those currently receiving antibiotics, nonsteroidal anti-inflammatory drugs, antidepressants, and/or appetite stimulants, or those exhibiting abnormalities such as high fever, icterus, dyspnea,

or anemia on clinical examination were excluded from the study. Based on the inclusion and exclusion criteria, a total of 50 cats of different ages, breeds, and sexes with FLUTD were enrolled following detailed anamnesis and physical examination.

Radiographic and Ultrasonographic Examinations

All cats with FLUTD findings underwent urinary bladder ultrasonographic examination with minimal stress and restraint, lasting no more than five minutes. The area was shaved, cleaned with alcohol, and gel was applied before using a 7.5 MHz microconvex transducer (Mindray Z60, China). Bladder integrity, wall thickness, and the presence of sediment and/or stones were assessed. Additionally, radiographic examination was performed in two laterolateral and ventrodorsal views of the abdominal region (Fujifilm CR, Japan).

Collection of Urine Samples, Urine Dipstick and Sediment Examinations

During ultrasonographic examination, urine samples (2–5 mL) were collected via ultrasound-guided cystocentesis using a 22G needle. Urine dipstick analysis (URIT-31) was performed within five minutes to assess pH, specific gravity (SG), leukocytes (WBC), and erythrocytes (RBC). The remaining urine samples (1–2 mL) were centrifuged at 1500 rpm for 10 minutes, and the sediment was examined under a light microscope ($\times 40$ magnification, Olympus, Japan) for the presence of crystalluria.

Forming Subgroups

The 50 cats included in the study were categorized into four subgroups based on the morphological characteristics of crystals detected in urine sediment examination: calcium oxalate (CaOx, $n=9$), hippuric acid ($n=8$), uric acid ($n=8$), and struvite ($n=25$).

Statistical Analyses

Statistical analyses were performed using SPSS version 27.0. The normality of continuous variables was assessed using the Shapiro-Wilk test. For non-normally distributed data, results were expressed as median (min–max). Descriptive statistics were calculated using the same software. Intergroup comparisons were conducted using the Kruskal-Wallis test for non-parametric data, with statistical significance set at $p < 0.05$.

Results

Anamnesis and clinical examination findings

The median duration of clinical signs in the cats included in the study was 3 days (range: 1–5 days). According to anamnesis data, all cats exhibited hematuria, stranguria, and dysuria, indicative of FLUTD. Stranguria was observed in all cats (50, 100%), while some cats (9, 18%) had both stranguria and hematuria, and the rest of the cats (41, 82%) had hematuria accompanied by stranguria.

Sex

Of the 50 cats included in the study, 28 were male (14 neutered, 14 intact), and 22 were female (13 neutered, 9 intact). In the CaOx group, 5 were male and 3 were female; in the hippuric acid group, 4 were male and 4 were female; in the uric acid group, 3 were male and 5 were female; and in the struvite group, 16 were male and 9 were female. Sex distribution of the cats is presented in Figure 1.

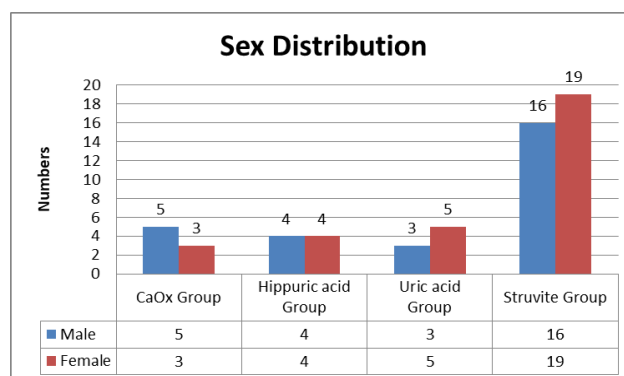


Figure 1. Sex distribution of the cats

Age

The median age of all cats included in the study was 3 (1-8) years. The median ages for each group were as follows: CaOx group, 4 (2-5.5) years; hippuric acid group, 2.5 (1-6.5) years; uric acid group, 3.25 (1-6) years; and struvite group, 3 (1-8) years. Age of the cats is presented in Figure 2.

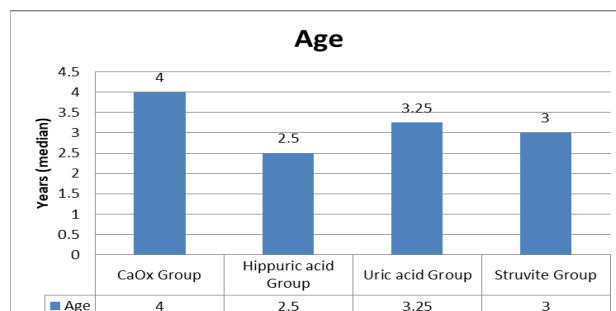


Figure 2. Age distribution of the cats

Body Weight

The median body weight of all cats included in the study was 3.75 (2.1-6) kg. The median body weights for each group were as follows: CaOx

group, 4 (2.5-5.75) kg; hippuric acid group, 3.1 (2.3-6) kg; uric acid group, 3.85 (3.2-5.6) kg; and struvite group, 3.75 (2.1-6) kg. Body weights of the cats are presented in Figure 3.

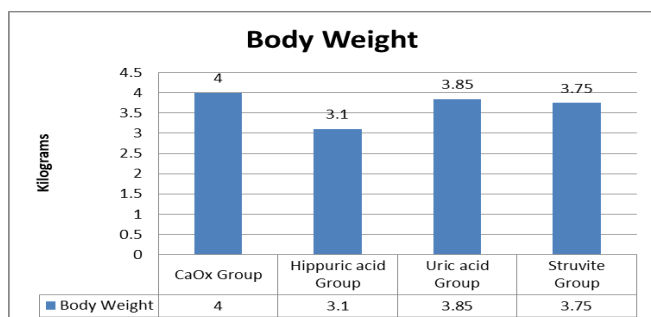


Figure 3. Body weight of the cats

Diet

Of the 50 cats included in the study, 35 were fed commercial dry food, while the remaining 15 were on a home-prepared diet. In the CaOx group, 7 cats were fed commercial dry food and 2 were on a home-prepared diet. All cats in the hippuric acid group (8 cats) were fed a home-prepared diet. In the uric acid group, 5 cats were fed commercial dry food and 3 were on a home-prepared diet. In the struvite group, 23 cats were fed commercial dry food, while 2 were on a home-prepared diet. Diets of the cats are presented in Figure 4.

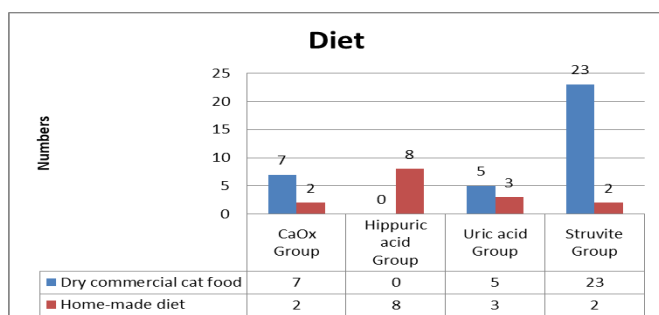


Figure 4. Diet of the cats

Breed

Among the 50 cats included in the study, 18 were Tabby, 8 were Exotic, 6 were Scottish, 1 was Persian, 10 were British, and 7 were Bombay. In the CaOx group, 2 were Tabby, 3 were Exotic, 1 was Scottish, 2 were British, and 1 was Bombay. In the hippuric acid group, 1 was Tabby, 3 were Scottish, 2 were Exotic, 1 was Bombay, and 1 was British. In the uric acid group, 3 were Tabby, 2 were Exotic, 1 was Scottish, 1 was British, and 1 was Bombay. In the struvite group, 12 were Tabby, 6 were British, 4 were Bombay, 1 was Scottish, 1 was Persian, and 1 was Exotic. Breed distribution of the cats is presented in Figure 5.

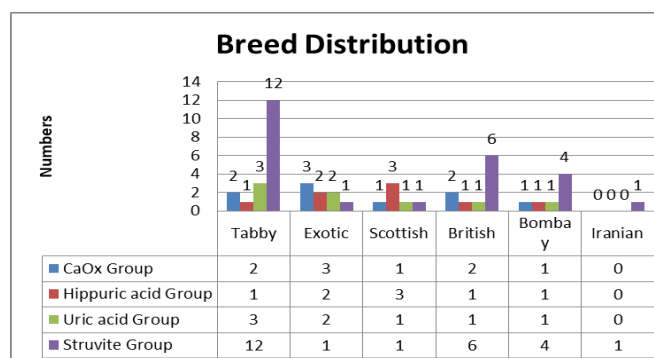


Figure 5. Breed distribution of the cats

Lifestyle

Among the 50 cats included in the study, 23 were indoor, while 27 were semi-indoor or had access to the outdoors. In the CaOx group, 6 were indoor and 3 were semi-indoor. In the hippuric acid group, 2 were indoor and 6 were semi-indoor. In the uric acid group, 3 were indoor and 5 were semi-indoor. In the struvite group, 12 were indoor and 13 were semi-indoor. Lifestyle of the cats is presented in Figure 6.

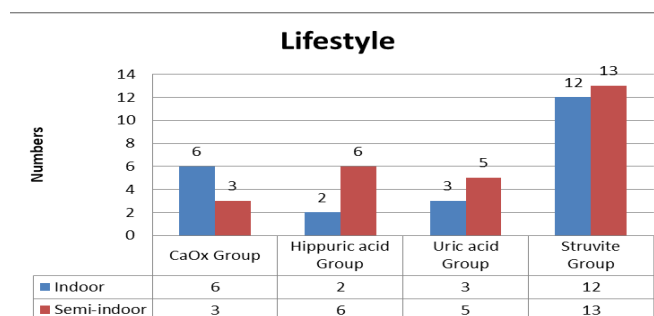


Figure 6. Lifestyle of the cats

Neuter Status

Of the 50 cats included in the study, 27 were neutered and 23 were intact. In the CaOx group, 3 cats were neutered and 6 were intact; in the hippuric acid group, 4 cats were neutered and 4 were intact; in the uric acid group, 6 cats were neutered and 2 were intact; and in the struvite group, 14 cats were neutered and 11 were intact. Neuter status of the cats is presented in Figure 7.

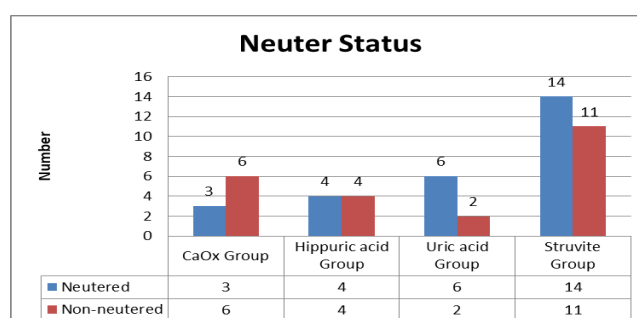


Figure 7. Neuter status of the cats

Adoption/Home-raised

Of the 50 cats included in the study, 19 were adopted from shelters or other homes, and 31 were born at home. Of the CaOx group, 3 were adopted and 6 were born at home; of the hippuric acid group, 2 were adopted and 6 were born at home; of the uric acid group, 4 were adopted and 4 were born at home; and of the struvite group, 10 were adopted and 15 were born at home. Origin of the cats is presented in Figure 8.

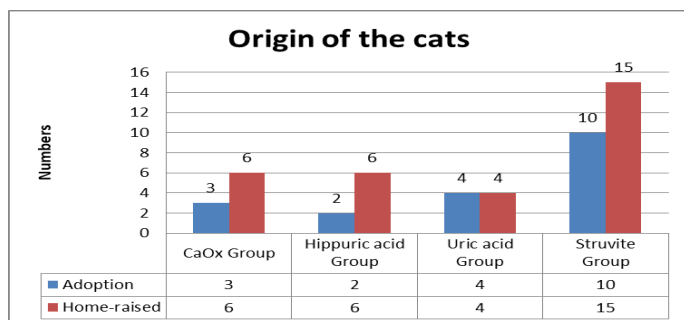


Figure 8. Origin of the cats

Breeding Status

Of the 50 cats included in the study, 10 had a history of mating, while 40 had never mated. In the CaOx group, 1 cat had mated before, and 8 had never mated. In the hippuric acid group, 1 had mated before, and 7 had never mated. In the uric acid group, 1 had mated before, and 7 had never mated. In the struvite group, 7 had mated before, and 18 had never mated. Breeding status of the cats is presented in Figure 9.

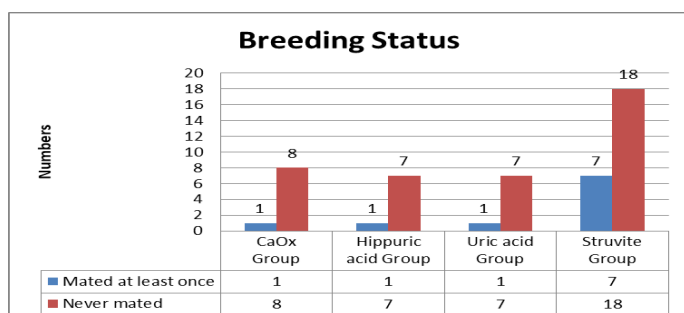


Figure 9. Breeding status of the cats

FLUTD Findings

All cats in the CaOx group exhibited periuria and stranguria, with hematuria present in 7 cats and dysuria in 2 cats. Similarly, all cats in the hippuric acid group had periuria and stranguria, accompanied by hematuria in 4 cats and dysuria in 4 cats. In the uric acid group, all cats also exhibited periuria and stranguria, with hematuria observed in 7 cats and dysuria in 1 cat. In the struvite group,

all cats had stranguria, which was accompanied by pyuria in 6 cats, periuria in 19 cats, dysuria in 2 cats, and hematuria in 23 cats. FLUTD findings of the cats are presented in Figure 10.

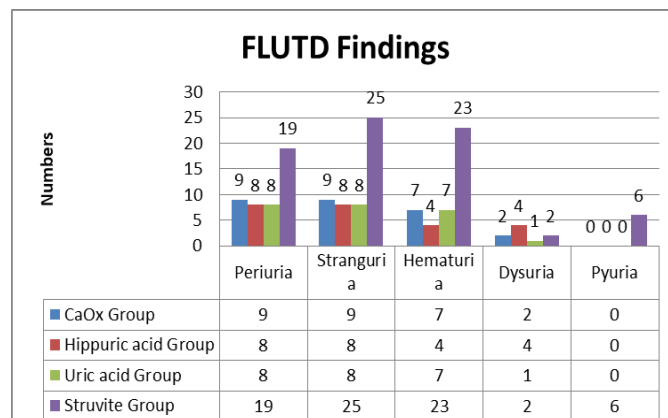


Figure 10. FLUTD findings of the cats

Urine dipstick analysis results

For all cats included in the study, the median urine pH was 6.75 (4–8.5), and the median urine specific gravity (Sg) was 1.030 (1.030–1.040). The urine pH of the CaOx group was 5 (4.5–6.5), with a urine Sg of 1.035 (1.030–1.040). In the hippuric acid group, the urine pH was 5.5 (5–6) and the urine Sg was 1.032 (1.030–1.040). The uric acid group had a urine pH of 4.25 (4–5) and a urine Sg of 1.035 (1.030–1.040). In the struvite group, the urine pH was 8 (7–8.5) and the urine Sg was 1.035 (1.030–1.040). While there was no significant difference in urine Sg levels ($p = 0.616$), urine pH levels varied significantly, with the lowest values in the CaOx group and the highest in the struvite group ($p < 0.0001$).

Discussion

Crystalluria is defined as the presence of crystals in the urine, typically resulting from the oversaturation of certain electrolytes and substances. The key factors triggering crystalluria include the accumulation of calcium oxalate, urate, and phosphate ions, which are excreted by the kidneys and exceed the urine's dissolving capacity. In the presence of crystalluria, crystals can become trapped in the matrix, leading to plug formation. While severe crystalluria can result in urethral obstruction even in the absence of a colloid matrix, it often remains subclinical or causes mild clinical signs in many cats. However, when persistent and severe, crystalluria can contribute to urolith formation, ultimately leading to urethral

obstruction and cystitis. In this study, cats with FLUTD findings were examined for crystalluria and categorized based on crystal type, with their demographic data evaluated comparatively. Struvite was identified as the most common crystal type. The cats with crystalluria were, on average, over 3 years old, had a body weight of 3.5 kg, were male, consumed commercial dry food, were of tabby breed, neutered, had never mated, and had access to the outdoors (semi-indoor). Considering these demographic characteristics could aid in identifying at-risk cats, enabling early screening and preventive measures to reduce further health complications.

Typical findings associated with FLUTD include pollakiuria, periuria, stranguria, and/or hematuria (Black, 2018). Clinical examination may reveal pain in the pelvic region and thickening of the urinary bladder wall on abdominal palpation, while other physical examination findings are usually unremarkable (Stella et al., 2011; Buffington and Chew, 2013). In cats with non-obstructive FIC that is not complicated by other diseases, serum biochemistry and CBC findings remain within normal limits. Therefore, urine analysis plays a crucial role in diagnosis. Hematuria and proteinuria are commonly detected, while pyuria or bacteriuria may be present but are not pathognomonic. The prevalence and severity of crystalluria vary; in a study of 62 cats with non-obstructive FIC, crystalluria was reported in 50% of affected cats (Kruger et al., 1991; Kruger et al., 1996). Urine in these cases is typically acidic and concentrated (Kruger et al., 1996). In the present study, when cats were categorized based on crystal type, all cats in the CaOx group exhibited periuria and stranguria, with hematuria detected in 7 cats and dysuria in 2 cats. Similarly, all cats in the hippuric acid group had periuria and stranguria, with hematuria in 4 cats and dysuria in 4 cats. In the uric acid group, periuria and stranguria were also present in all cats, accompanied by hematuria in 7 cats and dysuria in 1 cat. In contrast, all cats in the struvite group exhibited stranguria, with pyuria in 6 cats, periuria in 19 cats, dysuria in 2 cats, and hematuria in 23 cats. These variations in clinical findings may be influenced by multiple factors, including environmental temperature, neurological influences, diet, and cats' stress responses to different environments (Cannon and Alstadt, 2015).

The ideal urine pH range for cats is 6.2–6.5. When pH deviates from this range, the risk of ammonium phosphate and calcium oxalate crystal formation increases, directly contributing to crystalluria (Naarden and Corbee, 2020). In this study, the median urine pH of all cats was 6.75 (range: 4–8.5), while the median urine specific gravity (Sg) was 1.030 (range: 1.03–1.040). Urine Sg reflects the kidney's ability to concentrate or dilute urine, depending on the body's hydration status and renal function. A normal urine Sg for cats typically falls between 1.020 and 1.040. Higher values indicate concentrated urine, which is often seen in healthy cats but may also suggest dehydration or conditions that promote crystal formation. Lower values can indicate renal dysfunction or excessive water intake (Kruger et al., 1996). Consistent with previous reports, struvite crystals were associated with higher urine pH, while calcium oxalate and uric acid crystals were linked to more acidic urine (Buffington and Chew, 2013). In the CaOx group, urine pH was 5 (4.5–6.5) and urine Sg was 1.035 (1.030–1.040). In the hippuric acid group, urine pH was 5.5 (5–6) and urine Sg was 1.032 (1.030–1.040). In the uric acid group, urine pH was 4.25 (4–5) and urine Sg was 1.035 (1.030–1.040). In the struvite group, urine pH was 8 (7–8.5) and urine Sg was 1.035 (1.030–1.040). While no statistically significant difference was observed in urine Sg levels ($p > 0.616$), urine pH was significantly lower in the CaOx group and highest in the struvite group ($p < 0.0001$). These findings highlight the critical role of urine pH and Sg in crystalluria. Maintaining an appropriate dietary regimen can help regulate urine composition, reducing the risk of urolithiasis and crystal formation. Since urine Sg indicates urine concentration, monitoring this parameter alongside pH can provide valuable insights into hydration status and metabolic health. Dietary and fluid intake adjustments should be key considerations for cats at risk (Westropp et al., 2019).

Non-obstructive FLUTD affects male and female cats equally, although the risk is higher in neutered animals. Non-obstructive FIC is most commonly observed in cats aged 2–6 years that have lived exclusively indoors and have been fed dry food. Comorbid metabolic diseases, such as obesity, are also associated with FIC (Buffington, 2011; Chew and Buffington, 2013). Relapses occur in 17.1–65% of cats with FIC, and the incidence of relapse

decreases with increasing age (Kruger et al., 2003; Kaul et al., 2019). In the present study, the median age of all cats included was 3 years (range: 1–8). The median age of the CaOx group was 4 years (2–5.5), the hippuric acid group was 2.5 years (1–6.5), the uric acid group was 3.25 years (1–6), and the struvite group was 3 years (1–8). Additionally, the median body weight of all cats was 3.75 kg (2.1–6). The median body weight of the CaOx group was 4 kg (2.5–5.75), the hippuric acid group was 3.1 kg (2.3–6), the uric acid group was 3.85 kg (3.2–5.6), and the struvite group was 3.75 kg (2.1–6). Although the mean ages of the cats in this study were consistent with previous reports, none of the cats were clinically overweight or obese. However, at ages and body weights where the incidence of FLUTD increases (Kaul et al., 2019), performing at least urine dipstick and urine sediment examinations in cats with similar demographic characteristics may help prevent further complications. These diagnostic tools offer the advantages of being cost-effective and providing rapid results, allowing for the timely initiation of treatment and management programs (Buffington, 2011).

The urine volume of cats fed exclusively dry food is 40% lower than that of cats fed only wet food. Reduced urine volume may lead to increased urine concentration, which can aggravate urothelial damage. As a result, neurogenic inflammation begins, accompanied by crystalluria (Kruger et al., 2015). In this study, the median urine pH of all cats was 6.75 (range: 4–8.5), and the median urine Sg was 1.030 (range: 1.03–1.040). Additionally, it was determined that 35 out of 50 cats were fed commercial dry food, while the remaining 15 were fed a home-prepared diet. In the CaOx group, 7 cats were fed commercial dry food and 2 were fed a home-prepared diet. In the hippuric acid group, all 8 cats were fed a home-prepared diet. In the uric acid group, 5 cats were fed commercial dry food and 3 were fed a home-prepared diet. In the struvite group, 23 cats were fed commercial dry food, while 2 were fed a home-prepared diet. These findings can be explained by the low urine volume observed in cats fed only commercial dry food and the urothelial damage, inflammation, and crystalluria that may result from the concentrated urine produced under these conditions (Kruger et al., 2003; Kruger et al., 2015; Kaul et al., 2019). Moreover, due to the variability and unmonitored nature of home-prepared diets in semi-indoor cats, dietary intake could not be precisely assessed (Kruger et al., 2015). Moreover, these findings

align with the understanding that a major cause of FLUTD is a homogeneous diet, an imbalance in the calcium-phosphorus ratio, and excessive magnesium intake, all of which contribute to urine crystallization (Lekcharoensuk et al., 2001).

Although cats with FLUTD may not exhibit detectable clinical signs, underlying abnormalities can affect urothelial integrity, bladder permeability, glycosaminoglycan secretion, adrenal function, and the central nervous system. There may be no direct correlation between clinical findings, cystoscopic bladder appearance, and histological changes. Histopathological findings often include urothelial damage, submucosal hemorrhage and edema, submucosal vasodilation with marginal neutrophils, increased mast cell infiltration, fibrosis, and an elevated sensory nerve fiber count (Buffington and Chew, 2017). FLUTD encompasses various conditions affecting the bladder and urethra in cats, with the type of urinary crystals playing a significant role in clinical presentation and disease severity. Unlike calcium oxalate uroliths, struvite stones can often be dissolved through dietary management, making early detection particularly beneficial. Struvite crystalluria can lead to urolith formation, causing frequent urination, straining to urinate, hematuria, and inappropriate urination outside the litter box. In severe cases, particularly in male cats, urethral obstruction may develop, requiring immediate veterinary intervention (Piyarungsri et al., 2020). Calcium oxalate crystals generally form in acidic to neutral urine and are not directly linked to dietary magnesium levels. These crystals can aggregate into uroliths, producing clinical signs similar to struvite stones, including hematuria, dysuria, and potential urinary obstruction. Unlike struvite stones, calcium oxalate uroliths do not dissolve with dietary management and typically require surgical removal (Lund et al., 2013). Ammonium urate crystals, though less common, may develop in cats with metabolic disorders or liver diseases. These crystals contribute to urolith formation, leading to hematuria and dysuria. Diagnosis and treatment generally involve addressing the underlying condition predisposing the cat to ammonium urate crystalluria (Defauw et al., 2011). Beyond toxicological contexts, hippuric acid crystals are rarely observed in feline urine. Their formation is influenced by dietary factors, particularly the ingestion of substances metabolized into benzoic acid, which conjugates with glycine to form hippuric acid. However, as typical feline diets contain minimal amounts of these precursors, hippuric acid crystalluria remains uncommon in healthy cats (Kramer et al., 1984). In summary, urinary crystal type significantly

influences the clinical manifestations of FLUTD. Identifying the specific crystal type is essential for selecting an appropriate treatment strategy, which may involve dietary modifications, increased water intake, medications, or surgical intervention. The crystalluria patterns observed in this study, except for hippuric acid crystals, align with previously reported predisposing factors. The presence of hippuric acid crystals in this study may be attributed to dietary variation. These findings highlight the importance of regular veterinary check-ups and urinalysis for early crystalluria detection and management, ultimately reducing the risk of severe urinary tract complications in the feline population of the region (Dorsch et al., 2014).

In this study, 50 owned cats presented to the Harran University Veterinary Faculty Animal Hospital between March 2024 and January 2025 with clinical signs suggestive of FLUTD were systematically evaluated through comprehensive physical and laboratory examinations, with a primary focus on crystalluria. Struvite was the most frequently detected crystal type ($n = 25$), followed by calcium oxalate (CaOx, $n = 9$), hippuric acid ($n = 8$), and uric acid ($n = 8$). Demographic analysis indicated that cats with crystalluria were predominantly male, neutered, tabby, and three years of age or older, with an average body weight of 3.5 kg. Most were fed commercial dry food, had never mated, and exhibited a semi-indoor lifestyle. The association between crystalluria and these demographic factors reinforces the influence of diet and environmental conditions on urinary health in cats. Given the potential for crystalluria to progress to more severe complications, such as urethral obstruction, early identification and targeted interventions—including dietary modifications and routine urinalysis—are essential for effective management. These findings highlight the need for further research into the role of dietary composition, hydration strategies, and lifestyle factors in the pathophysiology and prevention of feline lower urinary tract disorders.

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Research Article

Investigation of Thiol Disulphide Balance in Dogs with Canine Leishmaniasis

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Abstract

Previous studies have demonstrated that oxidative stress plays a significant role in the etiopathogenesis of diseases. In this study, it was aimed to elucidate the etiopathogenesis of Canine Leishmaniasis by comparing the levels of thiol/disulfide, known as oxidative stress markers, in affected dogs and clinically healthy controls. Specifically, the objective was to determine the levels of dynamic Thiol-Disulfide Homeostasis in serum, including native thiol, total thiol, and disulfide, as well as to calculate the ratios of disulfide/total thiol and disulfide/native thiol.

A total of 30 dogs aged between 1 and 9 years were included in the study. These dogs were divided into two groups: positive and negative, to form the experimental and control groups. Blood samples were collected from dogs suspected of having leishmaniasis in veterinary clinics (Caniv-4 Leishmania, Vet Expert, Vet Planet Ltd., Poland). Using a diagnostic kit screening for four infectious diseases, dogs that tested positive only for leishmaniasis and negative for the remaining three were included in the infected group, while dogs that tested negative for all were included in the control group. Serum samples were stored at -20°C and analyzed in the laboratory for thiol, disulfide, and ischemia-modified albumin (IMA) levels.

Thiol-disulfide and IMA levels of dogs diagnosed with leishmaniasis (n=20) and healthy dogs (n=10) were compared. Native thiol and total thiol levels in leishmaniasis-positive dogs were found to be similar to those of healthy controls. The findings suggest that oxidative stress may occur in dogs with Canine Leishmaniasis when thiol-disulfide homeostasis is used as a reference marker.

Keywords: Canine leishmaniasis, Thiol-disulfide homeostasis, Oxidative stress, Ischemia-modified albumin

Introduction

Canine leishmaniasis (CanL), caused by *Leishmania* spp. parasites, is a significant zoonotic disease that can lead to severe systemic infections in dogs. This disease is endemic in regions such as the Mediterranean basin, South America, and certain parts of Asia, and it is transmitted through the bite of infected female sandflies belonging to the *Phlebotomus* genus (Ready, 2014). CanL may manifest within a broad clinical spectrum ranging from cutaneous lesions to systemic organ involvement, and if left untreated, it can result in fatal outcomes.

Leishmaniasis presents in three main clinical forms: visceral (VL), cutaneous (CL), and mucocutaneous (MCanL). Approximately 1.3 million new cases are reported annually, with around 300,000 of these being VL cases, while the remaining cases consist of CanL and MCanL (WHO, 2013).

In the pathogenesis of the disease, the host's immune response plays a crucial role. In host cells, the production of reactive oxygen species (ROS) increases in response to infection, which facilitates the elimination of parasites. However, excessive ROS production can also lead to oxidative stress and tissue damage (Persson et al., 2014). Oxidative stress is defined as an imbalance between the rate of production of reactive oxygen and nitrogen species (ROS/RNS) and the antioxidant defense capacity (Yi & Khosla, 2016).

The thiol-disulfide balance is an important biochemical parameter in the evaluation of oxidative stress. Thiols maintain cellular redox homeostasis by neutralizing free radicals. Oxidation of thiol groups results in the formation of disulfide bonds, reflecting the organism's oxidative stress burden (Erel & Erdoğan, 2020). In this process, antioxidants prevent the formation of ROS or repair the damage caused, thereby playing a critical role in the organism's defense mechanisms (Valko et al., 2007).

Alterations in thiol-disulfide homeostasis have been closely linked to disease severity and prognosis in various infectious conditions, including Canine Leishmaniasis (CanL). Oxidative stress, resulting from excessive production of reactive oxygen species (ROS), can contribute to cellular dysfunction and suppression of the immune system. Emerging

evidence suggests that the thiol-disulfide balance may serve as a critical biomarker for monitoring the clinical course of infectious diseases (Halliwell & Gutteridge, 1984).

The present study aims to investigate the role of thiol-disulfide homeostasis in dogs diagnosed with CanL, evaluating its association with oxidative stress and clinical outcomes. Furthermore, serum levels of ischemia-modified albumin (IMA) were measured to explore their potential impact on disease progression. The overall objective was to enhance understanding of the pathophysiological mechanisms involved and to support the development of more effective diagnostic and therapeutic strategies in the management of Canine Leishmaniasis.

Materials and methods

Ethical Approval

This study was conducted with the approval of the Kırıkkale University Local Animal Experiments Ethics Committee (approval number: 2025-01/06).

Animal Material

The animal material used in this study consisted of dogs presented to veterinary clinics located in Bodrum, Muğla province in the Aegean region of Turkey. Informed consent was obtained from all owners of the animals included in the study. A total of 30 dogs were used: 20 dogs diagnosed with CanL and 10 clinically healthy dogs, varying in breed, sex, and age (between 1 and 9 years).

Blood Sample Collection

Blood samples were collected from the cephalic vein of the participating dogs into anticoagulant-free tubes and allowed to clot for 20 minutes at room temperature. Following this, the samples were centrifuged at 3000 rpm for 10 minutes, and sera were separated. The obtained sera were tested using a rapid diagnostic kit (Caniv-4 *Leishmania*, Vet Expert, Vet Planet Ltd., Poland) for *Leishmania*, *Ehrlichia*, *Anaplasma*, and *Dirofilaria* infections. Dogs that tested positive only for *Leishmania* and negative for the other three pathogens were included in the CanL group, while those testing negative for all four were assigned to the control group. Sera designated for analysis were stored at -20°C until testing.

Laboratory Analyses

Once sampling was completed, the thawed sera were analyzed in the laboratory. Native thiol (NT),

total thiol (TT), and disulfide (D) levels were measured using the method described by Erel and Neşelioğlu (2014). Ischemia-modified albumin (IMA) levels were determined using the method proposed by Bar-Or et al. (2000).

Statistical Analysis

The variables were examined with the Shapiro-Wilk test and Levene test as parametric test assumptions. The differences in NT, TT, Disulfide, Disulfide/NT, Disulfide/TT, NT/TT and IMA among the groups were analyzed using Student t test. Receiver operating characteristic (ROC) analysis was used to determine a predicted threshold for identification of canine Leishmaniasis. ROC curves for the detection of canine Leishmaniasis was obtained for the parameters that were found to be statistically significant. Sensitivity, specificity, 95 % confidence interval, and area under the curve (AUC) were calculated for the parameters. Differences with $p < 0.05$ were considered statistically significant. All statistical analyses were performed using IBM SPSS 23.0 and MedCalc Version 9.2. The comparison of serum native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol ratios, and ischemia-modified albumin (IMA) levels between dogs diagnosed with Leishmaniasis

and clinically healthy (control group) dogs is presented in Table 1. The results indicated that native thiol and total thiol levels were significantly lower in dogs with Leishmaniasis ($p < 0.05$), while changes in the other parameters were not statistically significant ($p > 0.05$).

Results

Among the control group dogs, 4 (40%) were female and 6 (60%) were male. In the Leishmania group, 11 (55%) were female and 9 (45%) were male. The youngest dog in the control group was 2 years old, and the oldest was 8 years old, with a mean age of 4.7 years. In the Leishmania group, the youngest was 2 and the oldest 12 years old, with a mean age of 5.6 years. The control group consisted of 10 dogs, 5 of which were mixed-breed, while the others were terrier (1), pointer (1), cavalier king charles (1), doberman (1), and dogo argentino (1). The positive group (CanL) included 7 mixed-breed dogs and 13 purebred dogs, including beagle (1), husky (1), pointer (2), rottweiler (1), golden retriever (2), terrier (1), labrador retriever (2), chihuahua (1), schnauzer (1), and yorkshire terrier (1).

It was observed that 60% of the control dogs had a regular vaccination schedule. Among the

Table 1 : Comparison of native thiol, total thiol, disulfide levels, their ratios, and IMA levels between dogs with Leishmaniasis and healthy (control) dogs

Parameter	Group	Mean	Std Error	Std Dev.	Median	Minimum	Maximum	p
NT	Control	250,53	16,55	52,35	255,50	168,90	317,00	0,014*
	Leish	205,97	8,84	39,54	210,00	132,10	256,40	
TT	Control	318,07	19,87	62,83	337,45	220,50	396,60	0,019*
	Leish	265,20	11,36	50,82	274,20	168,20	327,80	
Disulfide	Control	33,77	2,18	6,89	33,05	24,80	42,55	0,115
	Leish	29,62	1,44	6,44	30,45	18,05	41,05	
Disulfide/NT	Control	13,64	0,62	1,97	13,55	10,79	16,20	0,290
	Leish	14,40	0,39	1,74	14,08	11,77	18,20	
Disulfide/TT	Control	10,68	0,39	1,22	10,66	8,88	12,23	0,278
	Leish	11,15	0,23	1,04	10,99	9,53	13,34	
NT/TT	Control	78,64	0,77	2,45	78,68	75,53	82,25	0,278
	Leish	77,69	0,47	2,08	78,03	73,32	80,95	
IMA	Control	0,77	0,02	0,06	0,76	0,68	0,86	0,873
	Leish	0,77	0,02	0,07	0,76	0,66	0,92	

Control: Control group; Leish: Leishmaniasis group; NT: Native thiol; TT: Total thiol; IMA: Ischemia-Modified Albumin.

*: Significant difference between groups ($p < 0.05$).

In the assessment conducted in terms of oxidative stress, a significant difference was observed between the groups for total thiol and native thiol levels. Based on these differing values, ROC curve analysis was performed (Table 2). When comparing total and native thiol levels, total thiol was found to be more specific in evaluating leishmaniasis in infected dogs (Figure 1, Figure 2).

Table 2: Comparison of total thiol and native thiol levels using ROC curve analysis

Parameter	Threshold	Se	%95 CanL for Se	Sp	%95 CanL for Sp	AUC	p
NT	$\leq 246,8$	85,00	62,1 - 96,6	70,00	38,4 - 93,0	0,765	0,008
TT	$\leq 327,8$	100,00	83,0 - 100,0	60,00	26,4 - 87,6	0,765	0,008

NT: Native thiol, TT: Total thiol



Figure 1: ROC curve comparison of native thiol and total thiol measurements in dogs with Leishmaniasis.

Leishmania-positive dogs, 13 (65%) had mixed breeding and 6 (30%) were selected from shelters, while 10% were identified as street-origin dogs. In contrast, all dogs in the control group (30%) were reported to be raised in home. In the Leishmania group, 10 dogs (50%) were also reported to be raised and were living in gardens.

Dogs in the control group weighed between 4 and 45 kg, with an average body weight of 23.3 kg. Dogs in the Leishmania group weighed between 3–40 kg, with an average weight of 22.3 kg. In the control group all except for one dog(10%) received regular external anti-parasitic treatment. Whereas only 9 dogs(45%) in the Leishmania group were reported to undergo regular external parasitic treatment. 13(65%) of the dogs in the Leishmania group were reported of not having regular anti-parasitic drug treatment.

As shown in Table 1, when comparing serum levels of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol, and ischemia-modified albumin between dogs with leishmaniasis and healthy controls, it was found that native and total thiol levels were significantly

lower in dogs with Leishmaniasis ($p < 0.05$). No statistically significant differences were found in the other measured parameters ($p > 0.05$).

Discussion

Canine leishmaniasis (CanL) is recognized as one of the most significant vector-borne zoonotic diseases affecting dogs worldwide (Dantas-Torres et al., 2012). Its chronic and multisystemic course, characterized by a broad spectrum of clinical manifestations, complicates both diagnosis and management (Mann et al., 2021; Baneth et al., 2021). The most frequently reported clinical findings include dermatological lesions and localized or generalized lymphadenomegaly (Cardoso et al., 2021). The clinical diagnosis of Canine Leishmaniasis (CanL) remains particularly intricate, given that approximately half of the infected canine population exhibit no overt clinical symptoms (Mancianti et al., 1988). Moreover, the clinical presentation is marked by considerable heterogeneity and lacks specificity. Typically, the disease manifests as a chronic, multisystemic condition with potential involvement of multiple organ systems throughout the body (Solano-Gallego et al., 2011).

Due to its unique geographical and climatic

characteristics, Turkey is considered an endemic region for CanL, where the disease has evolved into a notable public health concern (Toz et al., 2013). Reported prevalence rates across different regions of Turkey vary significantly, ranging from 0% to 37.4% (Aslan Çelik et al., 2019). Epidemiological data have demonstrated regional disparities in CanL prevalence across Turkey: a seroprevalence of 0% has been reported in Southeastern Anatolia, approximately 2% in Central Anatolia, and the highest burden in the Aegean region, with a documented prevalence of 37.4% (Aydenizöz et al., 2010; Aslan Çelik et al., 2019; Bakırcı and Topçuoğlu, 2021). The present investigation was accordingly conducted in the Aegean region, recognized as the most endemic area for CanL within the country.

This study aimed to evaluate alterations in thiol-disulfide homeostasis (TDH) in dogs diagnosed with CanL, with a specific focus on the role of oxidative stress in disease pathogenesis. The findings demonstrated a statistically significant decrease in native thiol (NT) and total thiol (TT) levels in infected dogs, whereas the increase in disulfide levels was not statistically significant. This observation may be interpreted as indicative of thiol reservoir exhaustion, potentially attributable to the heightened oxidative stress burden experienced by the affected organisms.

The pathogenesis of CanL involves an immune response triggered by the intracellular replication of *Leishmania* spp. within macrophages, which in turn leads to increased production of reactive oxygen species (ROS). However, excessive ROS production can induce oxidative damage to host cells and impair immune function (Persson et al., 2014; Yi & Khosla, 2016). During this process, thiol groups are oxidized into disulfide bonds, thereby disturbing the physiological thiol-disulfide equilibrium (Erel & Erdogan, 2020).

Numerous veterinary studies have investigated the oxidative stress induced by various infectious diseases through the evaluation of TDH parameters. Çamkerten et al. (2019) reported significantly reduced NT, TT, and disulfide levels in sheep with sarcoptic mange, although disulfide/NT, disulfide/TT, and NT/TT ratios showed no significant difference between the infected and control

groups. These results suggest that oxidative stress in sarcoptic mange is accompanied by a decrease in antioxidant capacity. In alignment with these findings, Değirmençay et al. (2021) reported a significant perturbation in the thiol-disulfide homeostasis among distemper virus-infected canines. Similarly, Terzi et al. (2021) documented decreased concentrations of total thiol (TT) and native thiol (NT) in diarrheic calves. Collectively, the converging evidence from these three clinical investigations underscores a consistent biochemical trajectory associated with systemic inflammatory conditions. In another investigation, Kurtdele et al. (2022) demonstrated that asymptomatic dogs infected with canine parvovirus (CPV) exhibited increased levels of malondialdehyde (MDA), paraoxonase-1 (PON-1), and disulfide, accompanied by reduced NT and TT levels. In contrast, Şenel et al. (2024) reported that NT and TT levels were higher in symptomatic CPV-infected dogs compared to controls. These data suggest that in acute pathologies such as canine parvoviral enteritis (CPV), particularly those progressing with sepsis, endogenous antioxidant defense mechanisms may be mobilized at earlier stages of disease progression. Furthermore, the elevated levels of antioxidant markers such as TT and NT during convalescence—when compared to control subjects—could imply an active role of redox homeostasis in the recovery cascade. Contrastingly, in the current study, dogs afflicted with CanL exhibited significantly diminished TT and NT levels, reflective of a pronounced oxidative stress status. In the present study, the significant decrease in NT and TT levels among dogs with CanL indicates a state of oxidative stress. While these results diverge from those of Şenel et al. (2024), they are in agreement with findings from other chronic disease models. It is therefore plausible to suggest that the oxidative stress profile in CanL resembles that observed in chronic infections such as sarcoptic mange rather than in acute, rapidly progressing diseases like CPV.

Ehrlichia, Anaplasma, and Dirofilaria infections have also been shown to trigger oxidative stress responses in dogs. In a recent study by Dokuzeylül et al. (2024), it was reported that dogs infected with Anaplasma and Ehrlichia had significantly increased total oxidant capacity (TOC) and nitric oxide (NO) levels, along with reduced total

antioxidant capacity (TAC), indicating oxidative stress. Elevated oxidative stress index (OSI) levels in these vector-borne diseases support the concept of disrupted redox homeostasis. These findings are in agreement with the current study, which also suggests an imbalance in oxidative status related to *Leishmania* infection.

In another study by Gültekin et al. (2017), oxidative stress markers were evaluated in CanL-positive dogs, and a significant reduction in the enzymatic antioxidant PON-1 activity was observed compared to healthy dogs ($p < 0.001$), although no significant difference in total antioxidant capacity (TAC) levels was found. The results of the current study are consistent with those of Gültekin et al., confirming the presence of oxidative stress in dogs with CanL. This study also demonstrated through ROC analysis that NT and especially TT levels could serve as potential biomarkers in the diagnosis of CanL. The high sensitivity and specificity of TT levels support their use as potential diagnostic indicators. In line with previous literature, decreased thiol levels and increased oxidative stress load were observed in CanL-positive dogs (Halliwell & Gutteridge, 1984; Valko et al., 2007). However, ischemia-modified albumin (IMA) levels showed no statistically significant difference between groups, suggesting that IMA may not be a specific marker for CanL. Total oxidant and antioxidant capacity, ceruloplasmin and ischemia-modified albumin (IMA) are some of the important biomarkers of oxidative stress (Ateş et al., 2016; Elmas et al., 2017; Hanikoğlu et al., 2016; Erel and Neşelioğlu 2014). Ischemia-modified albumin is an oxidant marker which is a derivative of albumin (Lippi and Montagnana, 2009). In human medicine, many studies are available on the relationship between IMA and increased oxidative stress (Özcan et al. (2018).

They indicated that the levels of IMA were positively correlated with other oxidant parameters (Ds, Ds/NT, and Ds/TT) and negatively correlated with antioxidant parameters (NT, TT, and NT/TT). (Değirmencay et al. (2021). In a 2021 publication on oxidative stress in dogs with distemper, they found that IMA was higher in dogs with distemper compared to the control group (Değirmencay et al., 2021). In the study conducted by Terzi et al. in 2023 with calves, a total of 50 calves were

included in the study. There were 25 calves in both the diarrheal and healthy groups. In neonatal diarrheal calves, natural thiol ($P=0.025$) and total thiol ($P=0.041$) values were significantly decreased compared to the healthy control group. Disulfide ($P=0.133$), disulfide/natural thiol ratio ($P=0.001$) and IMA ($P=0.0018$) parameters were lower in the healthy group and the difference between the two groups was significant for parameters other than disulfide. This study shows that TDH is impaired in neonatal calf diarrhea and IMA levels increase due to oxidative stress (Terzi et al., 2023). A negative correlation was observed between thiol and IMA in these two studies. In this study, no significant difference was detected in IMA levels between the infection and control groups.

Based on the findings, it has been proposed that preserving thiol-disulfide balance and reducing oxidative stress may be beneficial for clinical management of CanL. Antioxidant therapies containing thiol-based agents such as N-acetylcysteine (NAC) and alpha-lipoic acid (ALA) could provide therapeutic benefits in this context (Güler et al., 2021; Tunalı et al., 2022). Nevertheless, the study has certain limitations. The relatively small sample size and lack of inclusion of various disease stages may limit the generalizability of the results. Future studies involving a larger and more homogeneous population, including longitudinal designs, would allow for a more comprehensive evaluation of thiol-disulfide balance and other oxidative stress markers.

Conclusion

In conclusion, this study demonstrated that thiol-disulfide homeostasis is disrupted in dogs with CanL, and oxidative stress plays a central role in the pathogenesis of the disease. The observed reductions in NT and TT levels indicate that these parameters may serve as reliable diagnostic and prognostic biomarkers. Targeting oxidative stress and using appropriate antioxidant support may provide a promising therapeutic approach for CanL management. However, this approach needs to be validated through larger, randomized controlled trials.

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This manuscript reports the results of the same-name graduate study and has never been published in another place.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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Research Article

Prevalence of Systemic Hypertension in Dogs and Plasma Cystatin C as a Biomarker for Target Organ Damage Risk Assessment

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Abstract

This study aimed to determine the prevalence of systemic hypertension and the associated risk of target organ damage in dogs, while evaluating plasma cystatin C levels as a potential biomarker for early renal damage detection. A total of 93 dogs presented to the Small Animal Clinic, Faculty of Veterinary Medicine, Aydın Adnan Menderes University were included. Blood pressure measurements were performed using a non-invasive oscillometric device (petMAP Graphic II), and dogs were classified according to the American College of Veterinary Internal Medicine guidelines. Of the dogs evaluated, 61% were hypertensive, with 18% classified as prehypertensive, 23% as hypertensive, and 19% as severely hypertensive based on target organ damage risk. Plasma cystatin C levels were significantly higher in hypertensive dogs compared to normotensive dogs ($p<0.01$). Furthermore, cystatin C levels were notably elevated in the prehypertensive ($p<0.05$) and severely hypertensive ($p<0.001$) groups compared to the normotensive group. These results highlight systemic hypertension as a significant health concern in dogs and suggest that plasma cystatin C may serve as a reliable biomarker for early renal damage detection associated with hypertension. However, further studies with larger sample sizes and long-term monitoring are required to validate these findings.

Keywords: cystatin C, dogs, hypertension, target organ damage

Introduction

Systemic hypertension is a chronic condition prevalent in both humans and animals, often leading to significant target organ damage if left untreated (Brown et al., 2007; Acierno et al., 2018). Prolonged elevation of blood pressure beyond normal limits can result in severe health complications, affecting vital organs such as the heart, kidneys, eyes, and brain (Stepien et al., 2003). In veterinary medicine, diagnosing and managing systemic hypertension pose challenges, as the disease often remains asymptomatic until advanced stages, when irreversible damage may have already occurred (Acierno et al., 2018). Hypertension is broadly categorized into primary

(essential) hypertension and secondary hypertension. While the precise etiology of primary hypertension remains unclear, genetic predisposition and environmental factors are believed to play key roles (Mooney et al., 2017). In contrast, secondary hypertension typically arises from identifiable underlying causes, including renal diseases, endocrine disorders, and the use of certain pharmacological agents (Acierno et al., 2018).

Accurate blood pressure measurement is essential for diagnosing and monitoring hypertension in dogs. Non-invasive measurement techniques, widely favored in clinical settings, are considered reliable tools for

assessing target organ damage risk (Haberman et al., 2004; Stepień et al., 2003). However, relying solely on blood pressure values may not provide a complete picture of the pathological impacts of hypertension, highlighting the need for additional biomarkers to assess early organ damage (Brown et al., 2007).

In recent years, the use of biomarkers for the early detection of target organ damage associated with hypertension has gained significant attention. Among these, cystatin C has emerged as a reliable indicator for assessing glomerular filtration rate (GFR) and detecting early renal damage in hypertensive dogs. Studies have shown that serum cystatin C possesses greater sensitivity and specificity for diagnosing chronic kidney disease (CKD) compared to traditional markers like creatinine (Ko et al., 2021). Furthermore, cystatin C has demonstrated superior prognostic value in predicting mortality and cardiovascular morbidity in hypertensive populations, outperforming creatinine in clinical relevance (Carretero et al., 2018). Additionally, urinary cystatin C has shown promise as an early biomarker for detecting kidney disease and organ damage in dogs with hypertension (Sivasakthi et al., 2018). Its association with early kidney injury in critically ill dogs further emphasizes its utility in intensive care settings for monitoring renal function and identifying acute kidney injury (AKI) (Paes-Leme et al., 2021).

This study aimed to evaluate the prevalence of systemic hypertension in dogs and to assess the effectiveness of plasma cystatin C levels as a biomarker for hypertension-related target organ damage. The findings are expected to contribute to the early diagnosis and management of hypertension in dogs, facilitating the development of appropriate therapeutic strategies to improve clinical outcomes.

Materials and Methods

This study was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Approval No: 64583101/2019/086, dated 28.08.2019). Written informed consent was obtained from the owners of all participating dogs prior to their inclusion in the study. A total of 93 dogs of varying breeds, ages, and genders, presented to the Small Animal Clinic, Department of Internal Medicine, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, for examination or treatment between January 2020 and April 2021, were enrolled in the study.

Detailed anamnesis data were collected for each dog included in the study, encompassing information such as breed, age, gender, body weight, diet, medical and surgical history, current medications, antiparasitic treatments, and vaccination status. Clinical examination findings, including body temperature, heart and respiratory rates, skin elasticity, mucous membrane appearance, capillary refill time, heart auscultation results, and stress levels, were meticulously recorded. Additionally, complete blood count and serum biochemical analyses were performed, and radiological and echocardiographic findings were documented.

Dogs previously diagnosed with acute or chronic cardiac/renal failure, those on medications affecting blood pressure, those displaying stress-induced variations during measurements, or dogs for which reliable data could not be obtained through oscillometric blood pressure measurements were excluded from the study (Acierno et al., 2018).

The included dogs were classified based on the 2018 American College of Veterinary Internal Medicine (ACVIM) hypertension guidelines (Acierno et al., 2018). In the first classification, a systolic blood pressure (SBP) threshold of 140 mmHg was used, and dogs were categorized into normotensive and hypertensive groups. In the second classification, considering the risk of target organ damage, dogs were divided into four subgroups: normotensive, prehypertensive, hypertensive, and severely hypertensive.

Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate measurements were performed non-invasively using appropriately sized cuffs placed on the tail, front, and hind legs with an oscillometric device (petMAP Graphic II, Ramsey Medical, USA) (Vachon et al., 2014; Gloria et al., 2015). For each dog, a minimum of six repeated measurements were taken, and the average value was calculated to determine systemic blood pressure. To ensure measurement accuracy, dogs were kept calm, and proper attention was given to selecting cuff sizes appropriate to limb circumference and ensuring correct cuff placement. Additionally, arterial pulsation graphs were monitored throughout the measurement process, and

emphasis was placed on obtaining a clear and accurate oscillometric curve.

Blood samples were collected from the Vena cephalica antebrachii of each dog in a single 8 mL dose using heparinized tubes. The samples were then centrifuged at 3000 rpm for 15 minutes (Hettich, Germany) to separate the plasma portion and subsequently stored at -20 °C until analysis. Cystatin C levels were measured using a dog-specific ELISA kit (Canine Cystatin C ELISA Kit, Abbkine, China). The analysis was conducted following the manufacturer's protocol, and the results were evaluated with a microplate reader. Urea and creatinine levels were analyzed using commercial test kits (Randox, USA) with an autoanalyzer (RX Monaco, Randox, USA) via the colorimetric method.

Statistical analyses were performed using SPSS 22.0 software (SPSS Inc., USA). Data from normotensive and systemic hypertensive dog groups were compared using the Mann-Whitney U test. Data categorized based on target organ damage risk were analyzed using the Kruskal-Wallis test, followed by paired comparison methods for multiple group comparisons. The relationship between plasma cystatin C concentration and other parameters was evaluated using the Pearson correlation test. In all statistical analyses, a p-value of <0.05 was considered statistically significant.

Results

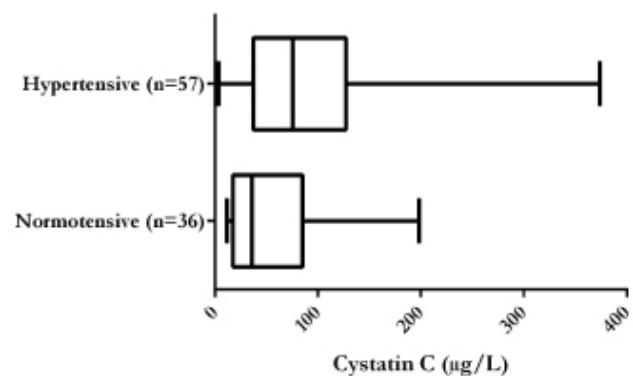
In this study, a total of 164 dogs presented to the Aydın Adnan Menderes University Faculty of Veterinary Medicine Research and Practice Hospital, Internal Medicine Small Animal Clinic for health check-ups, vaccinations, examinations, and treatments were initially evaluated. Following anamnesis and clinical examination, dogs with a history of diseases or medications that could affect the study data, those exhibiting significant stress reactions during blood pressure measurements, dogs for which artifact-free measurements could not be obtained in at least six repetitions, or those lacking an appropriate oscillometric curve were excluded from the study. Ultimately, 93 dogs meeting the study criteria were included, their data were systematically recorded, and detailed analyses were conducted.

The breed distribution of the 93 dogs included in the study was as follows: Mixed breed (n=41), Golden Retriever (n=6), Terrier (n=6), German Shepherd Dog (n=5), English Cocker Spaniel (n=4), Dobermann (n=4), Kangal (n=3), Staffordshire Bull

Terrier (n=3), Poodle (n=3), Pomeranian (n=3), Beagle (n=2), French Bulldog (n=2), Pointer (n=2), Pekingese (n=2), Weimaraner (n=2), Miniature Pinscher (n=2), Siberian Husky (n=1), Labrador Retriever (n=1), and Rottweiler (n=1). Of the included dogs, 32% (n=30) were clinically healthy with no laboratory abnormalities and were presented for routine vaccination and antiparasitic treatments. The remaining 68% (n=63) were brought to the clinic with various complaints, including weakness and loss of appetite.

In the evaluation, it was found that the systolic blood pressure (SBP) average exceeded 140 mmHg in 57 out of 93 dogs (61%), and these dogs were classified as hypertensive. Comparative analyses were conducted between normotensive and hypertensive dogs, focusing on age, gender, body weight, body temperature, heart and respiratory rates, systolic/diastolic/mean arterial blood pressure values, and plasma cystatin C, urea, and creatinine concentrations. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) values were found to be significantly higher in dogs with systemic hypertension compared to normotensive dogs ($p<0.001$). Additionally, plasma cystatin C concentration was significantly elevated in hypertensive dogs compared to normotensive dogs ($p<0.01$). However, no significant differences were observed between the two groups regarding plasma urea and creatinine concentrations ($p>0.05$). The comparative results of plasma cystatin C concentrations are summarized in Figure 1.

Figure 1. Plasma Cystatin C Concentrations in Systemic Hypertensive (SBP >140 mmHg) and Normotensive Dogs



Based on systolic blood pressure values and the risk of target organ damage, dogs were classified into four subgroups: normotensive (n=36), prehypertensive (n=17), hypertensive (n=22), and severely hypertensive (n=18). Comparative analyses were performed across these groups, evaluating age, gender, body weight, body temperature, heart and respiratory rates, systolic/diastolic/mean arterial blood pressure values, and plasma cystatin C, urea, and creatinine concentrations.

Significant differences were observed between the groups regarding systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and plasma cystatin C concentrations ($p<0.05$). Plasma cystatin C concentrations were significantly higher in the prehypertensive group ($p<0.05$) and the severely hypertensive group ($p<0.001$) compared to the normotensive group. Although cystatin C levels were elevated in the hypertensive group compared to the normotensive group, this difference did not reach statistical significance.

In addition, plasma urea concentrations were significantly lower in the prehypertensive group compared to both the normotensive and severely hypertensive groups ($p<0.01$). No significant differences were detected for other parameters among the groups. The distribution of plasma cystatin C concentrations according to target organ damage risk levels is shown in Table 1.

Correlation analyses revealed a moderate positive correlation ($r=0.495$) between plasma cystatin C concentration and body temperature in normotensive dogs, while a moderate negative correlation ($r=-0.387$) was observed with respiratory rate. No significant correlations were detected in systemically hypertensive dogs. When analyzed based on target organ damage risk levels, a moderate positive correlation ($r=0.673$) was found between plasma cystatin C concentration and age in the prehypertensive group. Additionally, a moderate positive correlation ($r=0.492$) was identified between plasma cystatin C concentration and heart rate in the hypertensive group.

Table 1. Demographic, Clinical, and Laboratory Findings of Dogs Classified by Target Organ Damage Risk Level

	Normotensive (SBP<140 mmHg; n=36)	Prehypertensive (SBP 140-159 mmHg; n=17)	Hypertensive (SBP 160-179 mmHg; n=22)	Severely hy- pertensive (SBP>180 mmHg; n=18)	P
Age	3,5 ± 2,9	3,9 ± 3,4	3 ± 2	5,6 ± 4,2	0,200
Gender (female/ male)	20/16	10/7	11/11	8/10	
Body weight (kg)	22 ± 13	15,2 ± 7,71	21,5 ± 9,5	17,6 ± 8,1	0,120
T (°C)	38,4 ± 0,4	38,6 ± 0,5	38,5 ± 0,3	38,6 ± 0,5	0,292
P (beats/min)	112 ± 13	110 ± 13	104 ± 18	119 ± 24	0,186
R (breath/min)	43 ± 13	46 ± 10	46 ± 13	49 ± 13	0,136
SBP (mmHg)	122 ± 10	148 ± 5	168 ± 4	194 ± 10	0,000
DBP (mmHg)	90 ± 14	93 ± 15	112 ± 13	113 ± 12	0,000
MAP (mmHg)	101 ± 11	112 ± 11	131 ± 9	140 ± 8	0,000
Cystatin C (µg/L)	52 ± 50a	89 ± 68b	64 ± 49ab	114 ± 81bc	0,008
Urea (mg/dL)	48 ± 16a	37 ± 16b	50 ± 26ab	89 ± 131a	0,039
Creatinine (mg/dL)	1,04 ± 0,2	1,06 ± 0,33	1,09 ± 0,37	2,0 ± 2,31	0,147

Discussion

In recent years, significant progress has been made in the diagnosis and management of hypertension in cats and dogs, yet current knowledge remains insufficient (Acierno et al., 2018). The widespread adoption of non-invasive blood pressure measurement devices in veterinary clinics has made identifying hypertension risk factors in feline and canine populations across various disease groups more accessible. However, blood pressure measurements are known to be susceptible to physiological fluctuations, and non-invasive devices may not provide results as precise as invasive measurements, which are considered the gold standard (Vachon et al., 2014; Gloria et al., 2015). Despite these limitations, non-invasive devices remain clinically valuable for identifying patients at risk of target organ damage (Acierno et al., 2018). Additionally, it has been emphasized that biomarkers are essential for distinguishing between transient or situational hypertension and pathological hypertension (Syme et al., 2020). This study aimed to measure systemic blood pressure in dogs using non-invasive methods, determine the prevalence of hypertension, and evaluate plasma cystatin C levels as a potential indicator of hypertension-related target organ damage. Hypertension was detected in 61% of the 93 dogs evaluated in this study, highlighting systemic hypertension as a significant health concern in dogs.

Studies investigating the epidemiology of hypertension in dogs remain limited in the veterinary literature compared to the extensive, large-scale studies conducted in humans (Mahmood et al., 2014; Kotchen et al., 2011). Over the years, threshold values for defining hypertension in dogs have been revised and updated. In the 2007 and 2018 guidelines published by the American College of Veterinary Internal Medicine (ACVIM), the normotension threshold was set at 140 mmHg (Brown et al., 2007; Acierno et al., 2018). It is reported that the prevalence of systemic hypertension can reach 100% in diseases associated with secondary hypertension, such as acute or chronic renal failure, hyperadrenocorticism, and diabetes mellitus (Acierno et al., 2018). However, this study did not focus on the etiology of hypertension in detail but rather analyzed the dogs' blood pressure values and biomarker levels. According to the findings, 18% of the dogs were

classified as prehypertensive, 23% as hypertensive, and 19% as severely hypertensive. These results suggest that hypertension and its associated target organ damage risk represent a considerable health issue within the canine population.

The effect of age and sex on blood pressure remains a controversial topic in the veterinary literature. While some studies report a positive correlation between age and blood pressure, suggesting that blood pressure tends to increase with age (Bodey and Michell, 1996; Bright and Dentino, 2002), others have failed to confirm this relationship (Mishina, 1997; Remillard, 1991; Meurs, 2000). Similarly, findings regarding the effect of sex on blood pressure are contradictory. Some studies suggest that male dogs have higher blood pressure values than females (Mishina, 1997; Bodey and Michell, 1996; Bright and Dentino, 2002), while others report no significant difference between sexes (Surman, 2012). In this study, age and sex were not found to have a statistically significant effect on blood pressure among dogs classified based on hypertension status and target organ damage risk. This observation aligns with findings from some previous studies (Mishina, 1997; Remillard, 1991; Surman, 2012). However, it is important to note that most of these studies were conducted on small sample sizes, and the absence of large-scale studies involving thousands of subjects, as seen in human medicine, remains a limitation in veterinary research.

The effect of breed on blood pressure has also been addressed in previous studies. It has been reported that hunting dogs, particularly Greyhounds, exhibit higher blood pressure levels and a higher prevalence of hypertension (Bright and Dentino, 2002). Conversely, certain breeds, such as Irish Wolfhounds, are noted to have relatively lower blood pressure values (Bright and Dentino, 2002). In this study, the breed information of each dog was recorded, but statistical analysis could not be performed due to an insufficient sample size for individual breeds. This limitation highlights the need for larger-scale studies that include sufficient representation of specific breeds to better understand the relationship between breed and blood pressure variability in dogs.

Accurate and reliable blood pressure measurement is essential for determining normotension and diagnosing hypertension in dogs. Although arterial

catheterization is considered the gold standard for blood pressure measurement, its use in routine clinical practice is limited due to the risk of complications and the need for specialized equipment (Acierno et al., 2018). In clinical settings, non-invasive oscillometric devices are preferred for their ability to provide quick and reliable measurements (Shih et al., 2010; Tebaldi et al., 2012; Da Silva et al., 2013; Vachon et al., 2014; Lyberg et al., 2021). The petMAP Graphic II device used in this study offered advantages such as arterial pulsation graph tracking, contributing to the acquisition of accurate and consistent measurement results.

It is well established that hypertension can cause target organ damage, primarily affecting the kidneys, eyes, brain, heart, and blood vessels (Acierno et al., 2018). While glomerular filtration rate (GFR) is considered the gold standard for evaluating renal function, routine clinical practice often relies on serum creatinine and urea levels. However, these parameters can be influenced by factors such as muscle mass, nutritional status, and body weight (Feeman et al., 2003; Parker and Freeman, 2011). In contrast, cystatin C has emerged as a more sensitive biomarker for assessing glomerular filtration rate (Laterza et al., 2002; Peralta et al., 2011). In human medicine, cystatin C is widely used for the early diagnosis of chronic kidney disease (CKD) and serves as a prognostic indicator of renal function (Hari et al., 2014; Wu et al., 2010). Its advantages over traditional markers highlight its potential for early detection of kidney damage in dogs with systemic hypertension.

In this study, plasma cystatin C concentration was found to be significantly higher in dogs with systemic hypertension compared to normotensive dogs ($p < 0.01$). Furthermore, when dogs were classified based on the risk of target organ damage, cystatin C levels were significantly elevated in the prehypertensive group ($p < 0.05$) and the severely hypertensive group ($p < 0.001$). These findings suggest that cystatin C may serve as an important biomarker for the early detection of hypertension-related kidney damage (Prats et al., 2010; Salgado et al., 2012; Zhao et al., 2020). However, the limited sample size and the single-time blood sampling approach in this study highlight the need for larger-scale studies with long-term follow-up to validate and expand upon these results.

Conclusion

This study reveals a high prevalence of systemic

hypertension in dogs and highlights plasma cystatin C as a promising biomarker for the early detection of hypertension-related renal damage. Elevated cystatin C levels, particularly in prehypertensive and severely hypertensive groups, suggest its clinical value in assessing target organ damage risk. While non-invasive blood pressure measurement remains essential, integrating cystatin C evaluation could improve early diagnosis and management. Larger-scale, long-term studies are needed to confirm these findings and enhance their clinical applicability.

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Research Article

Prognostic Value of Serum C-Reactive Protein (CRP), Procalcitonin, Fibrinogen, and D-Dimer Levels in Dogs with Parvoviral Gastroenteritis

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Abstract

This study investigated the prognostic value of various biomarkers in 20 puppies with parvoviral gastroenteritis. After treatment, 13 dogs recovered, and 7 died during the treatment process.

Clinically, general condition disorders such as loss of consciousness and fainting have been associated with increased mortality. Hematologically, marked leukopenia and lymphopenia were observed in deceased dogs, indicating the suppressive effect of parvovirus on the immune system.

Among the biomarkers examined, fibrinogen and D-dimer levels were the most reliable indicators with high accuracy (AUC = 1.00) in mortality prediction. CRP and procalcitonin levels did not differ significantly between the surviving and deceased groups.

A significant decrease in treated and recovered dogs, especially in D-dimer and aPTT levels, was detected, indicating an improvement in the coagulation system.

Fibrinogen and D-dimer are reliable biomarkers to predict disease severity and mortality risk in dogs with parvoviral enteritis. In contrast, CRP and procalcitonin are not suitable for this purpose. Early monitoring of coagulation parameters can improve clinical management.

Key words: Canine Parvovirus (CPV), Prognostic Biomarkers, D-dimer Leukopenia, Hemorrhagic Gastroenteritis

Introduction

Parvoviral enteritis in dogs continues to be a major disease worldwide, with a morbidity rate of up to 100% and a mortality rate of 10% (Goddard and Leisewitz, 2010; Brady et al., 2012). It is a life-threatening disease that usually occurs in young dogs between 6 and 20 weeks of age and rarely affects adults. It generally presents as acute, fibrinous, necrotic, and hemorrhagic enteritis, and sometimes with myocarditis (Goddard and Leisewitz, 2010; Baştan, 2012).

Rottweiler, Doberman pinscher, Berger Allemand, American pit bull, Staffordshire terrier, Labrador retriever, Springer spaniel, Dachshund, and Yorkshire terrier breeds are more susceptible

to parvovirus enteritis (Glickman et al., 1985). Although the causes of racial sensitivity are not fully known, genetic characteristics are thought to play a role (Aktaş et al., 2011; Baştan, 2012). Parvoviral enteritis is twice as common in male dogs over six months of age as in females. The disease is more common in the summer than in the winter months (Houston et al., 1996). In addition to dogs, wolves, hyenas, and foxes from the canid family can also get the disease (Öcal and Ünsüren, 2009).

Deaths from parvoviral enteritis are due to bacteremia and endotoxemia.

Otto et al. in 2000 reported that 82% of dogs with CPV enteritis had a measurable endotoxin in the

circulation. Endotoxin initiates a procoagulant response on endothelial cells through cytokines (e.g., tumor necrosis factor). The first response to endotoxin is the activation of coagulation. But tests that measure clotting time in vitro depend mainly on clotting factor deficiency or factor inhibition, so this response cannot be well characterized. Systemic hypercoagulability may evolve to hypocoagulability as the inflammatory response progresses, and disseminated intravascular coagulation (DIC), known as DIC, may produce signs of fulminant bleeding (Otto et al., 2000). Within a few hours following infection, concentrations of acute-phase proteins (APP) such as haptoglobin, serum amyloid A (SAA), and C-reactive protein (CRP) increase.

Prothrombin time, activated partial thromboplastin time, D-dimer, fibrinogen levels, anti-thrombin III activity, and platelet count are all factors to be considered for DIC evaluation. It is noted that bone marrow depression and gastrointestinal hemorrhage may occur in CPV infections. However, it is thought that the cause of hemorrhagic enteritis in CPV may be due to endotoxemia of coliform bacteria and an increase in cytokine levels (Er & Ok, 2015).

The D-dimer, which can be measured in whole blood and plasma, is a biomarker for fibrin production and breakdown. D-dimer levels are very low in healthy animals, but can rise rapidly in the case of thrombosis. Currently, the estimation of D-dimer levels is mainly used for the diagnosis of venous thromboembolism (VTE). In addition, they are frequently used to determine the ideal duration of anticoagulation and to monitor disseminated intravascular coagulation (DIC) in patients undergoing venous thrombosis (VTE) (Başer et al., 2022). CRP and Procalcitonin are biomarkers used in infectious and non-infectious inflammatory diseases. High levels of CRP in dogs with CPV infection have been associated with mortality.

This study aimed to investigate the prognostic value of Serum C-Reactive Protein (CRP), procalcitonin, antithrombin, fibrinogen, and D-dimer levels in dogs with Parvoviral Gastroenteritis, to determine the value of these markers in the prognosis of the disease and to guide the treatment.

Materials and Methods

This study was approved by Kırıkkale University Animal Experiments Local Ethics Committee (Approval No:39, date: 15.11.2023).

The animal material of the study consisted of 20

and 10 healthy puppies of different breeds and genders, ranging in age from 4 to 12 weeks, who were brought in with complaints of weakness, loss of appetite, vomiting, and diarrhea. A group of 20 dogs diagnosed with CPV infection using rectal swab samples and the Canivet® CPV Ag Test (Vet Diagnostik) commercial rapid test kit and a control group of 10 puppies aged 4-12 weeks who had no disease complaints, had a negative Parvo rapid test kit and were found to be healthy at examination. CPV-infected dogs are divided into two groups: dogs that recover and those that die based on their response to treatment. Physical and clinical examinations of all dogs were performed and recorded.

To determine the hematological and biochemical parameters, 4 ml blood samples were taken from the vena cephalica of puppies to EDTA tubes for hematological analysis, 4 ml to citrate tubes for aPTT, PT, D-dimer, and fibrinogen measurements, and 4 ml to non-anticoagulant tubes for serum. Hematologic analyses were performed using the Hasvet Mindray BC-5000 Vet device, aPTT, PT, D-dimer, and fibrinogen measurements were measured using the BCS XP Siemens device, Procalcitonin ELISA method (BIO-TEK EL X 800 and BIO-TEK EL X 50 devices), and CRP immunoturbidimetric method using the MINDRAY-BS400 device.

All puppies diagnosed with CPV infection were treated according to a standard treatment protocol. Cefazolin sodium, Metronidazole, Vitamins K and B, Metochlopromide, pantoprazole, isotonic sodium chloride, dextrose, and Ringer's lactate were used to maintain fluid and electrolyte balance.

For statistical analysis, the subjects were categorized into three groups: control (healthy), recovered patients, and deceased patients. Gender, race, general condition, rapid test result, and treatment outcome values in patients admitted to the clinic are given in the form of a frequency table. The normality of the data obtained in the study was evaluated by examining the Shapiro-Wilk and Kruskal-Wallis tests and the Skewness and Kurtosis coefficients, and the control of the homogeneity of the data was provided by the Levene test. The effect of general condition on response to treatment at the time of admission to the clinic was tested by Fisher's precision analysis. The Welch ANOVA test was used to examine the blood parameters examined during admission to the

clinic (Fox and Weisberg, 2023). Games-Howell multiple comparison test was applied to determine the difference between the groups. In recovered patients, pre-treatment and intra-treatment blood parameters were examined with a matched t-test. To examine the effect levels of biomarkers, the Receiver Operating Characteristic (ROC) curve analysis was performed. In this analysis, the “Deceased” group was defined as a positive class, and the area under the curve (AUC) was calculated for each biomarker. ROC analysis was used to evaluate the power of each parameter to distinguish cases resulting in death at the level of sensitivity and specificity. All analyses were completed using Jamovi and R programs, and the margin of error was evaluated as $\alpha=0.05$ (Jamovi, 2024; R Core Team, 2024).

Results

Considering the gender distribution of the patients, there are 9 female and 11 male patients. 3 (33%) of females and 4 (36%) males died. No significant difference in survival by gender was observed ($p = 1.00$), indicating that gender was not a determining factor in survival. In terms of breed, 6 different breeds were identified, and the largest group was crossbred dogs (35%).

Although the patients who died were mostly Cane Corso and crossbreeds, there was no significant difference between the breeds included in the study in terms of mortality-recovery rates ($p = 0.403$). In terms of the general clinical situation, most of the patients (75%) presented with depression. The survival rate is higher in this group (10/15). On the other hand, all of the patients presenting with coma died (2/2). This difference was found to be very close to the limit of significance ($p = 0.069$), and this suggests that the general situation at the time of clinical admission may affect survival. It is observed that the prognosis of patients presenting with the depressive picture is better. Finally, according to the rapid test results, 13 of the 18 patients who were positive recovered, while two of the 2 patients who were negative died. Although a positive test result showed a higher rate of survival, this difference was not statistically significant ($p = 0.111$). However, this result is of limited significance as there were only two cases in the negative test group. The distribution of demographic and clinical variables according to the survival status of the individuals who make up the groups evaluated in the study is given in Table 1.

Table 1. Distribution of demographic and clinical variables by survival status

Variable	Result		Total
	Died	Healed	
Gender			
Female	3	6	9
Male	4	7	11
P			1.00
Breed			
Cane Corso	2	0	2
Cross-bred	3	4	7
Kangal Shepherd Dog	1	4	5
Belgian Shepherd Dog	1	2	3
Standard Schnauzer	0	2	2
Doberman	0	1	1
P			0.403
Situation			
Coma	2	0	2
Faint	0	3	3
Depression	5	10	15
P			0.069
Rapid Test			
Negative	2	0	2
Positive	5	13	18
P			0.111

According to the results of the analysis, a significant difference was observed between the groups in terms of leukocyte (WBC), neutrophil (NEU), and lymphocyte (LYM) values. In particular, WBC levels were significantly lower in the deceased group ($3.16 \pm 1.07 \times 10^9/L$), while it was highest in the control group ($12.62 \pm 0.59 \times 10^9/L$) ($P < 0.001$). Similarly, the NEU level was significantly lower in the deceased group ($P < 0.001$). In terms of LYM level, there was a significant difference between the dying group ($0.33 \pm 0.01 \times 10^9/L$) and the recovered group ($0.98 \pm 0.24 \times 10^9/L$) ($P = 0.013$). Hemoglobin (HGB) and

hematocrit (HCT) values were significantly higher in the deceased and recovered groups than in the control group ($P = 0.003$ and $P = 0.007$, respectively).

MCHC values also differed significantly between the groups, and significantly higher values were found in the recovered group (35.94 ± 0.37 g/dL) ($P = 0.01$). On the other hand, erythrocyte (RBC), mean erythrocyte volume (MCV), mean hemoglobin content (MCH), and platelet (PLT) levels did not show a statistically significant difference between the groups ($P > 0.05$). The values in terms of the blood parameters examined when they were brought to the clinic of the study evaluated groups are given in Table 2.

Table 2. Blood parameters of patients admitted to the clinic

Parameters	Control	Deceased	Recovered	P
WBC ($10^9/L$)	$12.622 \pm 0.5925c$	$3.156 \pm 1.0676a$	$8.192 \pm 1.347b$	<0.001
NEU ($10^9/L$)	$8.694 \pm 0.5453b$	$2.509 \pm 1.0653a$	$6.392 \pm 1.2225a,b$	<0.001
LYM ($10^9/L$)	$5.012 \pm 2.0489a,b$	$0.33 \pm 0.0138a$	$0.978 \pm 0.2365b$	0.013
RBC ($10^9/L$)	5.245 ± 0.5238	6.139 ± 0.1522	5.935 ± 0.1631	0.26
HGB(g/dL)	10.19 ± 0.5593 a	12.914 ± 0.6874 b	13.069 ± 0.3898 b	0.003
HCT(%)	$29.08 \pm 1.6803a$	$36.543 \pm 1.6456b$	$36.423 \pm 1.1274b$	0.007
MCV (fL)	61.26 ± 0.6784	59.5 ± 2.0012	61.446 ± 1.2728	0.703
MCH (pg)	20.39 ± 0.4466	21 ± 0.7877	22.062 ± 0.4399	0.061
MCHC (g/dL)	33.58 ± 0.5312 a	35.3 ± 0.5429 a,b	35.938 ± 0.3732 b	0.01
PLT ($10^9/L$)	315 ± 42.4992	365.714 ± 109.993	365.538 ± 39.9483	0.692

There is a statistical difference between groups bearing different labels in the same line ($P < 0.05$)

According to the results of the analysis, a significant increase in lymphocyte (LYM) levels was observed during the treatment process (baseline: 0.978 ± 0.237 ; end: 2.342 ± 0.325 ; $P < 0.001$). However, a statistically significant decrease was found in erythrocyte series parameters such as erythrocyte count (RBC) and hemoglobin level (HGB) ($P = 0.038$ and $P = 0.038$, respectively). Platelet count (PLT) tended to increase close to the limit of significance after treatment (baseline: 365.5 ± 39.9 ; end: 469.5 ± 55.8 ; $P = 0.053$). Other hematologic parameters, WBC, NEU, HCT, MCV, MCH, and MCHC values did not show a statistically significant difference between pre- and post-treatment ($P > 0.05$). The blood parameter values of the recovered patients, who were brought to the clinic and evaluated during the treatment, are given in Table 3.

PT sec. showed significant differences between the groups ($P = 0.033$). While the duration of PT was higher in the control group (9.35 ± 0.67 sec), it was shorter in the dying and recovered groups. A statistically significant difference was observed between the groups in terms of aPTT ($P = 0.003$). The duration of aPTT was significantly shorter (13.79 ± 0.41 sec) in the deceased group, while the recovered group remained intermediate in this respect. Fibrinogen levels differed significantly ($P < 0.001$) and were significantly higher in the dying (4.81 ± 0.28 g/L) and recovered (4.41 ± 0.32 g/L) groups than in the control group (2.41 ± 0.17 g/L). D-dimer levels also differed significantly between the groups ($P = 0.002$). The highest value was found in the deceased group (5167.38 ± 1751.42 ng/mL) and the lowest value was found in the control group (439.15 ± 38.57 ng/mL). On the other hand,

C-reactive protein (CRP) and procalcitonin levels did not show a statistically significant difference between the groups ($P > 0.05$). In addition to the changes in the treatment process, the biomarker levels observed in different groups (control, recovered, and death) in terms of clinical outcome were also analyzed comparatively and summarized in Table 4. Fibrinogen (AUC = 1,000) and D-dimer (AUC = 1,000) showed excellent discriminating performance, providing complete differentiation between both groups. The threshold values of these markers were determined as 3.744 g/L and 1595.62 ng/mL, respectively, and both showed 100% sensitivity and 100% specificity. CRP (AUC = 0.529) has low classification power,

with only 57% sensitivity and 70% specificity. This suggests that CRP plays a limited role in predicting mortality in this patient group. PT (AUC = 0.193) and aPTT.

When biomarker levels were examined before and after treatment in recovered patients, PT. There was a difference between the two measurements in terms of INRTS, aPTT AFS (sec), and D-dimer (ng/mL) levels. ($P < 0.001$ and $P = 0.038$, respectively). Other markers did not show a statistically significant variability during the treatment process ($P > 0.05$). Biomarker levels of patients who recovered during treatment were analyzed comparatively during and after admission to the clinic and summarized in Table 5.

Table 3. Examination of blood parameter values of recovered patients ($\pm$ SE)

Parameters	Initial Measurement	Final Measurement	P
WBC ($10^9/L$)	8.192 $\pm$ 1.347	10.97 $\pm$ 1.437	0.22
NEU ($10^9/L$)	6.392 $\pm$ 1.222	7.134 $\pm$ 1.22	0.719
LYM ($10^9/L$)	0.978 $\pm$ 0.237	2.342 $\pm$ 0.325	<0.001
RBC ($10^9/L$)	5.935 $\pm$ 0.163	5.543 $\pm$ 0.199	0.038
HGB(g/dL)	13.069 $\pm$ 0.39	12.169 $\pm$ 0.426	0.038
HCT(%)	36.423 $\pm$ 1.127	34.431 $\pm$ 1.1	0.102
MCV (fL)	61.446 $\pm$ 1.273	62.177 $\pm$ 1.003	0.335
MCH (pg)	22.062 $\pm$ 0.44	21.962 $\pm$ 0.354	0.737
MCHC (g/dL)	35.938 $\pm$ 0.373	35.346 $\pm$ 0.271	0.146
PLT ($10^9/L$)	365.538 $\pm$ 39.948	469.462 $\pm$ 55.823	0.053

Table 4. Biomarker values according to clinical outcome values ($\pm$ SE)

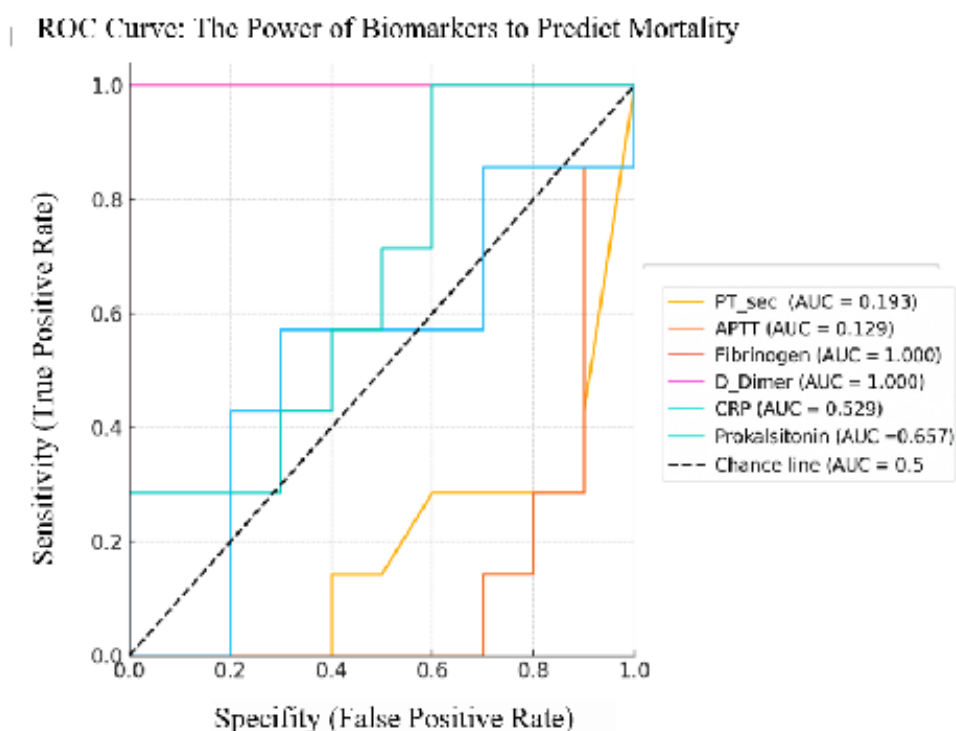
Biomarkers	Control	Deceased	Recovered	P
PT sec. TS(sec)	9.35 $\pm$ 0.670b	7.9 $\pm$ 0.476a,b	7.323 $\pm$ 0.044a	0.033
PT. INRTS	0.62 $\pm$ 0.01	0.643 $\pm$ 0.042	0.6 $\pm$ 0	-
aPTT AFS (sec)	20.3 $\pm$ 1.56b	13.786 $\pm$ 0.413a	17.885 $\pm$ 2.373a,b	0.003
Fibrinogen (g/L)	2.411 $\pm$ 0.165a	4.808 $\pm$ 0.277b	4.407 $\pm$ 0.324b	<0.001
D-dimer (ng/mL)	439.148 $\pm$ 38.569a	5167.381 $\pm$ 1751.421a,b	1549.261 $\pm$ 266.295b	0.002
C-Reactive Protein (mg/L)	0.806 $\pm$ 0.064	0.792 $\pm$ 0.131	0.638 $\pm$ 0.075	0.269
Procalcitonin(ng/mL)	815.317 $\pm$ 87.675	988.149 $\pm$ 103.842	891.55 $\pm$ 67.788	0.479

Table 5. Biomarker values in the convalescent group ($\pm$ SE)

Biomarkers	Initial Measurement	Final Measurement	P
PT sec. TS(sec)	7.323 $\pm$ 0.04408	7.515 $\pm$ 0.05975	0.760
PT. INRTS	0.6 $\pm$ 0	0.608 $\pm$ 0.00769	<0.001
aPTT AFS (sec)	17.885 $\pm$ 2.37306	14.2 $\pm$ 0.5051	<0.001
Fibrinogen (g/L)	4.407 $\pm$ 0.32439	1.946 $\pm$ 0.17358	1.000
D-dimèr (ng/mL)	1549.261 $\pm$ 266.29551	1363.903 $\pm$ 271.36166	0.038
C-Reactive Protein(m-g/L)	0.638 $\pm$ 0.0756	0.633 $\pm$ 0.07959	0.582
Procalcitonin(ng/mL)	891.55 $\pm$ 67.7883	844.752 $\pm$ 40.53414	0.771

(AUC = 0.129) did not exhibit significant predictive power with very low AUC values. While the highest specificity was achieved for these markers, the sensitivity was 0%; In other words, the correct

classification of death at these threshold values has failed. The predictive power of the biomarkers evaluated in the study was analyzed with ROC curves and AUC (Area Under the Curve); optimal cut-off, sensitivity, and specificity values were calculated (Figure 1).

**Figure 1.** AUC, cut-off, sensitivity, and specificity values of biomarkers in mortality discrimination

Discussion

Clinical manifestations observed in dogs with parvoviral enteritis include loss of appetite, depression, vomiting, bloody and foul-smelling diarrhea, dehydration, and fever are consistent with the classic symptoms described in the literature (Bird et al., 2013; Martin et al., 2002; Ulaş, 2015; Ince, 2017; Akdag, 2014). These findings directly reflect the severity of the infection, and it is characteristic that in the early stages of the disease, it starts with mild fever and depression, and soon develops into intense gastrointestinal symptoms. This picture has been confirmed in our cases.

In addition, findings such as impaired consciousness, hypothermia, and bradycardia progressing to coma in some cases were evaluated as signs of the development of sepsis and multiple organ failure. The survival rates we obtained are similar to the data reported in the literature. According to Melo et al. (2024), the development of systemic inflammatory response syndrome (SIRS) in CPV-2-infected dogs increases mortality by approximately 13 times. According to Paul et al. (2023), the mortality rate was significantly higher in dogs diagnosed with SIRS. This supports the relationship between mortality and severe clinical findings observed in our study. Loss of appetite, depression, vomiting, bloody and foul-smelling diarrhea, dehydration, and fever are consistent with the classic symptoms described in the literature (Bird et al., 2013; Martin et al., 2002; Ulaş, 2015; Ince, 2017; Akdag, 2014). These findings directly reflect the severity of the infection, and it is characteristic that in the early stages of the disease, it starts with mild fever and depression, and soon develops into intense gastrointestinal symptoms. This picture has been confirmed in our cases. In addition, findings such as impaired consciousness, hypothermia, and bradycardia progressing to coma in some cases were evaluated as signs of the development of sepsis and multiple organ failure.

Leukopenia, one of the most striking hematologic changes in dogs with parvoviral enteritis, is particularly in the form of neutropenia and lymphopenia, which is decisive for prognosis (Azetaka et al., 1981; Mylonakis et al., 2016). In our study, a significant decrease in WBC, neutrophil, and lymphocyte counts was found in the deceased group. These findings are in line with the data reported in a retrospective study of 401 cases by González-Domínguez et al. (2024). In this study,

the mean lymphocyte count was $0.82 \times 10^9/L$ in the deceased group and $1.27 \times 10^9/L$ in the surviving group. Similarly, in our study, the WBC value in the deceased group was approximately $3.2 \times 10^9/L$ and $12.6 \times 10^9/L$ in healthy controls. These data confirm that severe leukopenia, and especially lymphopenia, is decisive for prognosis has been determined. These findings indicate the suppressive effect of viral infection on bone marrow and lymphoid tissues and the weakness of the immune system (Castro et al., 2013; Kruse et al., 2010; Sykes, 2014).

A significant increase in the number of lymphocytes in the surviving dogs after treatment indicated that the immune system had recovered, and recovery had begun. In erythrocyte parameters, hemoconcentration due to dehydration was detected in the infected group; this led to significant increases in HGB and HCT. However, in advanced cases, blood loss through diarrhea and vomiting may have masked anemia (Gülersoy et al., 2022; Terzungwe et al., 2018).

In our study, CRP and procalcitonin levels were reported in a study conducted by McClure et al. (2013), where CRP levels reached an average of 88 mg/L at admission in puppies with parvovirus infection, and this value was significantly higher in fatal cases. In addition, Paul et al. (2023) showed in their study that combined models including MPV and chloride levels, as well as CRP, predicted mortality more effectively. In light of these findings, it is understood that CRP may be associated with sepsis or poor prognosis when it is above certain threshold values, but it may not be sufficient on its own. Although it increased in the infected group, it was observed that there was no significant difference between the deceased and the survivors. This suggests that these biomarkers may not be strong prognostic markers on their own for CPV (McClure et al., 2013; Dossin, 2019). However, in the literature, it is reported that the CRP/Alb ratio is correlated with the severity of infection and may be prognostically beneficial (Bozkurt et al., 2021; Cagnasso et al., 2023).

On the other hand, fibrinogen and D-dimer levels were strongly correlated with the severity of CPV infection. It is noteworthy that fibrinogen and D-dimer levels were found to be high in the

deceased group, similar to the threshold values in the ROC analyses reported in the study of Corda et al. (2023). In our analysis, the optimal cut-off point for fibrinogen was determined as 3.74 g/L and for D-dimer as 1596 ng/mL, and the AUC value for both parameters was found to be 1.00. These values were determined similarly in the study of Corda et al., and ≥ 3.5 g/L for fibrinogen and ≥ 1500 ng/mL for D-dimer were associated with DIC and poor prognosis.

These results show that the data in our study are consistent with other findings in the literature and that the prognostic discrimination power of both markers is quite high. Significantly higher levels are associated with the development of coagulopathy and DIC (Whitehead et al., 2020; Corda et al., 2023). In the ROC analysis, the AUC value for these two markers was found to be 1.00. This shows that the prognostic discrimination power is very high. In addition, the decrease in antithrombin levels promotes the development of hypercoagulability and DIC. These findings suggest that coagulation system markers play an important role in the prognosis determination of CPV infection.

Conclusion

This study revealed that the combined evaluation of clinical, hematologic, and biochemical parameters in puppies with CPV made important contributions to the determination of the disease prognosis. In particular, monitoring parameters such as fibrinogen, D-dimer, and antithrombin may be effective in early detection of patients with poor prognosis and in determining aggressive treatment strategies.

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Research Article

Investigation of Selected Trace Elements in Ovine Skin Disorders

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Abstract

This study aimed to determine the levels of certain trace elements in 30 sheep presented to Kırıkkale University Animal Hospital with complaints of skin problems and hair loss. Blood, skin, and hair samples were collected from the sheep for microbiological, parasitological, and biochemical analyses. No infectious or parasitic agents were detected in microbiological and parasitological examinations. Serum levels of zinc (Zn), copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), selenium (Se), and molybdenum (Mo) were quantitatively analyzed using an ICP-OES device. The results revealed that Zn, Cu, Mn, and Co levels were below the reference ranges, while Fe was within normal limits and Se was higher than expected. Mo levels were found to be very low. Consistent with existing literature, especially Zn and Cu deficiencies were strongly associated with impaired skin integrity and poor fleece quality in sheep. Additionally, Co deficiency, through its role in vitamin B12 synthesis, indirectly affects skin health. The main cause of these deficiencies was attributed to the flock being fed exclusively with commercial feed and not having access to grazing. These findings highlight the necessity of including trace element analyses in routine examinations for sheep exhibiting dermatological issues. Following appropriate mineral supplementation and dietary adjustments, the flock's skin condition and fleece quality improved significantly. In conclusion, this study emphasizes the critical importance of maintaining trace element balance in sheep nutrition to ensure animal welfare and optimize economic productivity in sheep breeding.

Keywords: Sheep, skin disease, trace element, zinc, copper, mineral deficiency.

Introduction

Skin and wool quality hold significant economic importance in sheep farming. Dermatological disorders in sheep not only compromise animal welfare but also have a substantial impact on wool production and its quality one of the primary outputs of the sheep industry. Both inadequate nutrition and trace mineral deficiencies are recognized as critical factors influencing wool growth.

Trace elements are required in minute quantities to support the normal progression of numerous biochemical activities and metabolic functions within the body. In sheep, essential trace elements include zinc (Zn), molybdenum (Mo), manganese (Mn), copper (Cu), iodide (I), iron (Fe), and cobalt (Co). These elements are vital for maintaining animal health and productivity, as they function as integral components of various enzymes and play regulatory roles in a wide range of biological processes (Al-Zubaidi et al., 2025; Swecker & Van Saun, 2023).

Zinc plays a pivotal role in numerous enzymatic processes and is one of the primary elements influencing the structure and function of the skin. It is also involved in keratinization, calcification, wound healing, as well as somatic and reproductive development. Dietary zinc deficiency, along with factors that interfere with its absorption or utilization, can lead to wool loss, wool-chewing behavior, and the development of parakeratosis in sheep (Zavosthi et al., 2012).

Copper deficiency in sheep can manifest primarily and sometimes exclusively through wool abnormalities. These abnormalities may include loss of crimp, a metallic sheen with increased luster in fur fibers, and a general deterioration of wool structure. Since copper is involved in the activity of enzymes essential for melanin synthesis, its deficiency is also associated with achromotrichia a condition characterized by the loss of fur pigmentation.

Photodermatitis, characterized by skin crusting and ulceration, may be observed in affected animals (Ebrahim et al., 2016). Cobalt is a key component of cobalamin (vitamin B12), which is essential for the formation of red blood cells and the maintenance of nervous tissue integrity. In ruminants, cobalt deficiency may lead to fatigue, reduced milk production, coarse and brittle fur or fleece, anemia followed by muscular weakness, impaired coordination, and an unsteady gait (Helmer et al., 2023).

There is no specific evidence in the literature indicating a direct effect of molybdenum on skin or wool health. However, excessive molybdenum intake can interfere

with copper absorption and bioavailability, potentially leading to secondary copper deficiency. As a result, signs associated with copper deficiency (such as coat depigmentation and wool abnormalities) may emerge indirectly (Helmer et al., 2023).

Iron is a critical structural component of hemoglobin and is essential for erythropoiesis. Iron deficiency anemia has been associated with low birth weight, preterm delivery, and increased oxidative damage in erythrocytes at the placenta–fetus interface. Although there is no clear evidence in the literature directly linking iron deficiency to skin or wool health, trace mineral imbalances can broadly affect animal physiology, potentially leading to general weakening, which may indirectly compromise coat quality. Furthermore, excessive iron intake may contribute to secondary copper deficiency (Moniello et al., 2010). Selenium, in conjunction with vitamin E, contributes to the antioxidant defense of organisms by serving as a component of the enzyme glutathione peroxidase. It also plays a crucial role in the immune system and in the metabolism of thyroid hormones, and is essential for reproduction. Given its importance in immune and metabolic functions, selenium deficiency may lead to general health issues such as weakness in lambs, which could indirectly impair coat quality. As for manganese, there is no specific evidence in the literature suggesting a direct impact on skin or wool health (Moniello et al., 2010).

Materials and Methods

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (Approval No: E.325808).

The study material consisted of 30 sheep of varying ages presented to the Animal Hospital of Kırıkkale University with complaints of skin disorders and fur loss. Anamnesis revealed that similar symptoms were observed across the entire flock. On-site examination indicated that the sheep were not allowed to graze, were raised in an open housing system, and were fed exclusively with commercial feed. From a total population of 250 animals on the farm, 30 sheep were selected for inclusion in the study.

From the 30 sheep included in the study, fur samples were collected for microbiological analysis and skin scrapings were obtained for parasitological examination. All animals underwent clinical examination for ectoparasites. Blood samples were collected from the clinically examined sheep into vacuum serum tubes. The samples were centrifuged

at 1500 × g for 10 minutes, and the resulting sera were stored at -20°C for the analysis of trace elements zinc, selenium, copper, manganese, cobalt, and molybdenum using inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroBlue, Germany).

Inductive Plasma Optical Emission Spectrometry Review

The analysis of trace elements was carried out using inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroBlue, Germany). Quantitative measurements of the analyzed elements were performed using the external calibration method based on calibration curves constructed from analytical standards at concentrations of 0, 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 ppm (Merck, Germany). The measurements of these analytical standards are summarized in Table 1.

Tissue sample digestion was performed using a microwave digestion system (CEM MARS 6), following the Blood Human and Animal Tissue methods. For sample pre-treatment (microwave digestion), 1 mL of serum was combined with 1 mL of 35% H₂O₂ and 5 mL of HNO₃. The mixture was subjected to digestion in the CEM MARS 6 microwave system under the following conditions: Power: 290–1800 W, Ramp time: 20:00 minutes, Hold time: 15:00 minutes, Temperature: 200°C. The final volume was adjusted to 15 mL before proceeding with the analysis.

The instrumental conditions used during ICP-OES measurements are summarized in Table 2.

Table 1. Wavelength and optical emission values of elements analyzed in the ICP-OES device.

Display Name:	BEC:	DL:	Std.Error:	Corr.Coeff	Range
Cu 224.700	47,5 ppb	1,300 ppb	1,26 ppb	0.99989	1,300 - 240 ppb
Co 228.616	22,2 ppb	0,063 ppb	0,156 ppb	0.99999	0,063 - 240 ppb
Se 196.090	140 ppb	4,060 ppb	3,81 ppb	0.99994	4,060 - 1200 ppb
Mn 257.611	4,79 ppb	3,750 ppb	2,58 ppb	0.99997	0,375 - 1200 ppb
Zn 226.5029	95 ppb	0,199 ppb	0.94 ppb	0.999760	199-120 ppb
Fe 238.204	19,5 ppb	0,493 ppb	3,38 ppb	0.99996	0.493 - 1200 ppb
Se 196.090	140 ppb	4,060 ppb	3,81 ppb	0.99994	4,060 - 1200 ppb
Mo 202.095	24,9 ppb	1,070 ppb	3,56 ppb	0.99995	1,070 - 1200 ppb

Table 2. ICP-OES instrument conditions.

Parameters	Value
Plasma Power	1435 W
Pump Speed	30 rpm
Coolant Flow	13 L/min
Auxiliary Flow	0,80 L/min
Nebulizer Flow	0,75 L/min
Number of replicates	3
Integration time (s) :	3 s
Sample uptake rate (μL/min)(speed)	0.3 rps

Table 3. Limit detection value for heavy metals

	Cu	Co	Zn	Fe	Mn	Se	Mo
Tespit Sınırı	1,300	0,063	0,071	0,493	0,375	4,060	1,070

Results

Field observations of the sheep included in the study revealed widespread fur loss across the entire flock. In some animals, wool loss was concentrated particularly around the neck and shoulder regions (Figures 1 and 2), with fibers shed at varying lengths. In several sheep, complete alopecia was noted in the dorsal and cranial regions, with a total absence of fur in these areas (Figures 3 and 4). Aside from dermatological findings, the general condition, feeding behavior, and mobility of the animals remained normal, and no pruritus (itching) accompanied the skin problems.



Figure 1. Fur loss localized in the neck and chest regions.



Figure 2. Fur loss of varying lengths observed in the neck and shoulder regions.



Figure 3. Severe alopecia observed in the cranial (head) region.



Figure 4. Alopecia involving the entire dorsal and cranial regions.

Table 4. Trace element levels in sheep

Element	mean	std	min	max	Normal serum level	Referans
Cu µg/ml	0,696667	0,180485	0,32	1,12	0.70–1.27 µg/ml	Laboklin.com, Underwood & Suttle (1999)
Co µg/ml	0,000714	0,00378	0	0,02	0.05 – 0.15 µg/ml	Radostits et al. (2007), Underwood & Suttle (1999)
Zn µg/ml	0,594667	0,13164	0,36	0,9	0.78–1.18 µg/ml	Laboklin.com, Underwood & Suttle (1999)
Fe µg/ml	2,175	0,833413	0,99	4,94	1.0 – 2.5 µg/ml	Laboklin.com, Underwood & Suttle (1999)
Mn µg/ml	0,038667	0,118489	0,01	0,66	0.08–0.14 µg/ml	McDowell (2003)
Se µg/ml	0,512	10,31382	0,38	0,57	0.04–0.08 µg/ml	Radostits et al. (2007), Underwood & Suttle (1999)
Mo µg/ml	0,001333	0,007303	0	0,04	0.05 – 0.15 µg/ml	Radostits et al. (2007), Underwood & Suttle (1999)

Discussion

The mean concentrations of the analyzed trace elements were as follows: copper (Cu) – 0.696667 µg/mL, zinc (Zn) – 0.594667 µg/mL, iron (Fe) – 2.175 µg/mL, manganese (Mn) – 0.038667 µg/mL, selenium (Se) – 0.512 µg/mL, and molybdenum (Mo) – 0.001333 µg/mL. Cobalt levels were found to be below the detectable limit. The average iron level of 2.175 µg/mL was within physiological reference ranges. Findings from the sampled animals revealed a strong correlation between reduced zinc and copper levels and the observed dermatological symptoms.

The findings of this study are consistent with the effects of zinc (Zn) deficiency on skin integrity, wound healing, and wool quality as described by Gade et al. (2023). Zn plays a critical role in keratin synthesis and maintaining epithelial integrity, while copper (Cu) is essential for melanin synthesis, enzymatic oxidation, and connective tissue stability (Underwood & Suttle, 1999; Stapay et al., 2023). Helmer et al. (2021) reported a direct association between reduced serum Cu and Zn levels and skin disorders in sheep. The immunological, epithelial, and keratinization-related functions of Zn and Cu have been well documented in the literature, and the current findings are in agreement with these reports (Radostits et al., 2007; Gade et al., 2023). Autukaitė (2023) reported that mineral deficiencies are more frequently observed in sheep flocks in Estonia during the winter season. Similarly, Silva et al. (2018) observed dermatological and wool abnormalities in animals with serum concentrations below Cu: 10.97 ± 3.61 µmol/L and Zn: 8.63 ± 2.22 µmol/L. Zavoshti et al. (2012) also associated zinc deficiency with dermatological symptoms in Makuui breed sheep.

Cobalt (Co) deficiency, particularly in ruminants, disrupts energy metabolism by impairing vitamin B12 synthesis and may indirectly affect skin health. Therefore, undetectable Co levels may serve as an important indicator for identifying subclinical cases. On the other hand, the inability to detect Co concentrations suggests a profound deficiency of this trace element. Cobalt is an essential precursor for vitamin B12 synthesized by rumen microorganisms, and its deficiency is associated with weight loss, reduced appetite, anemia, and decreased performance (Radostits et al., 2007).

In our study, the mean iron (Fe) level was determined to be 2.175 µg/mL, falling within the established reference range, while the mean selenium (Se) concentration of 0.512 µg/mL was found to be

higher than standard reference values. Selenium (Se) and molybdenum (Mo) are considered critical trace elements for maintaining skin health in sheep. In a case of molybdenosis reported by Helmer et al. (2021), excessive Mo intake was shown to disrupt copper metabolism, indirectly contributing to deficiencies in both selenium and cobalt. This imbalance was associated with reduced wool quality and the presence of dermatological lesions. Majak (2006) emphasized the safety limits of Mo as a feed additive, warning about the risk of copper deficiency with excessive Mo intake. He examined in detail the influence of pasture and feed composition on Se and Mo levels, highlighting that high Mo content can negatively impact skin health due to its antagonism with copper (Silva et al., 2018). In the present study, Mo levels were low, and therefore, the observed copper deficiency was not attributed to molybdenum imbalance.

In the examined flock, the fact that the animals were exclusively fed with commercial feed, had no access to grazing, and showed negative results in both microbiological and parasitological analyses—along with decreased serum levels of Cu, Zn, Mn, and Mo—combined with widespread skin disorders and fur loss in nearly all animals, suggested that the problem might have originated from the feed.

Following changes in the feeding regimen and the supplementation of the deficient trace elements, the dermatological symptoms in the flock were resolved.

Conclusion

In this study, significantly reduced levels of zinc (Zn), copper (Cu), and manganese (Mn) were detected in sheep presenting with skin disease. The inability to detect cobalt (Co) concentrations indicates a severe deficiency. These findings highlight the importance of incorporating trace element analysis into routine clinical evaluations. Mineral supplementation strategies should be individualized according to the animal's age, physiological status, and environmental conditions.

Treatment protocols should be based not only on clinical symptoms but also on trace element analyses. The findings of our study are supported by existing literature and emphasize the need for regular monitoring of trace element levels. Routine serum trace element analysis is crucial for developing feeding strategies tailored to specific rations and the individual mineral requirements of animals.

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Evaluation of Renal Functions in Cats with Cardiomyopathy

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Abstract

This study was carried out to evaluate the effects of heart failure due to cardiomyopathy on renal function in cats. For this purpose, physical examination, haematological and biochemical analyses, radiographic, electrocardiographic and echocardiographic imaging and cases were systematically investigated. The study material consisted of 25 cats in two different groups, the study group consisting of cats with cardiomyopathy (n:15) and the control group consisting of healthy cats (n:10).

N-terminal pro B-type natriuretic peptide (NT-proBNP) levels were significantly increased in cats with cardiomyopathy ($P<0.05$) and this increase was significantly correlated with left atrial diameter (LA), ventricular wall thickness (LVWF) and pulmonary artery flow velocity (PAVmax) determined echocardiographically (ECHO). In the renal parameters, especially SDMA was found to reflect the early renal effects than creatinine and BUN, which are classical renal markers, and an increase in SDMA values was found ($P<0.05$). In addition, SDMA showed significant correlations with some erythrogram and thrombogram parameters, suggesting its effects on haematopoiesis.

In conclusion, in this study, it was observed that cardiomyopathy-induced renal functions were affected early and significantly in cats. The use of biomarkers such as NT-proBNP and SDMA integrated into classical methods is important for early diagnosis and development of appropriate treatment strategies.

Keywords: Cardiomyopathy, Cat, Heart Failure, Kidney Damage, NT-ProBNP, SDMA.

INTRODUCTION

The heart and kidney are two vital organs that play a central role in maintaining the homeostatic balance of the organism. The physiological interaction between these two organs can turn into a bidirectional cycle of deterioration in pathological conditions. An acute or chronic dysfunction in the heart or kidney causes the functions of the other organ to be negatively affected (Bilgiç et al., 2012). In the clinical practice of veterinary medicine, especially in

cats, cardiac and renal diseases are frequently observed simultaneously, which necessitates a multidisciplinary approach. Identification of cardiac and renal damage, grading according to the International Renal Interest Society (IRIS) and development of appropriate treatment strategies accordingly are of great importance in terms of both prognosis and success of patient management (IRIS, 2023). With this study, it was aimed to determine renal involvement in cardiac patients, to reveal its place in the

context of veterinary medicine, especially in feline patients, and to provide a comprehensive evaluation based on the literature for the management of cardiac and renal involvement. Therefore, this study provides the basis for an in-depth examination of the anatomy and physiology of the heart and kidney and understanding the pathological interaction between these two systems. In this context, structural and functional classification of the heart, stages of heart failure according to the American College of Veterinary Internal Medicine (ACVIM) system, common cardiomyopathies in cats: Hypertrophic cardiomyopathy (HCM), hypertrophic obstructive cardiomyopathy (HOCM), restrictive cardiomyopathy (RCM), dilated cardiomyopathy (DCM) and unclassified cardiomyopathy (UCM), and the effects of cardiac failure on the kidneys were systematically analysed. This suggests an overlap between cardiovascular and renal disease. The haemodynamic interactions of heart and kidney failure, the impact on arterosclerotic disease in both organs and neurohormonal activation are some of the few examples of overlap (Bulletin, 2014).

In this study, various biochemical, hormonal and imaging-based diagnostic parameters were used to demonstrate the presence and clinical significance of cardiomyopathy-induced renal involvement in cats. Specific and general biochemistry indicators such as Symmetric Dimethyl Arginine (SDMA), Blood Urea Nitrogen (BUN) and Creatinine (CRE) and sodium-potassium-chlorine (NA-K-CL) were analysed to evaluate renal function. These parameters were used to determine early and late renal dysfunction by reflecting the glomerular filtration rate (GFR). N-terminal pro B-type natriuretic peptide (NT-proBNP) level, which is a diagnostic and prognostic indicator of cardiomyopathy, was preferred to evaluate myocardial tension and fluid overload. Radiologically, vertebral heart score (VHS) was calculated by thoracic radiography, thus the presence of cardiomegaly was objectively determined. In addition, structural abnormalities of the heart (left atrial enlargement, ventricular hypertrophy and systolic function) were evaluated by echocardiographic (ECHO) examinations. This multifaceted diagnostic approach allowed both the description and classification of the clinical picture of cardiomyopathy and renal involvement. In addition, neurohormonal activation, cytokine response, renin angiotensin aldosterone (RAAS) action, oxidative stress and central nervous system (SNS) mediated physiopathological processes were examined in detail.

One of the most prominent reactions in this process is characterised by an increase in sympathetic nervous

system (SNS) activity and a marked decrease in parasympathetic (vagal) tone. However, the renin-angiotensin-aldosterone system (RAAS) is activated and antidiuretic hormone (ADH) release increases (Sarıl, 2021). These neurohormonal changes initially show compensatory effects in heart failure states. The aim is to correct acute hypotension and hypovolemia by increasing body fluid volume and vascular tone. Although compensatory mechanisms contribute to restoring circulatory balance in the short term, the situation is reversed when they are activated for a prolonged and sustained period. The load on the heart increases, the vascular system deteriorates and a chronic cycle of decompensation develops. Therefore, neurohormonal responses, which are beneficial at first, become detrimental over time and negatively affect kidney function as well as the cardiovascular system (Sarıl, 2021).

In conditions such as heart failure, volume overload causes an increase in central venous pressure. This increase leads to congestion in the renal veins, an increase in intra-abdominal pressure and consequently a decrease in glomerular filtration rate (GFR). Decreased GFR causes further activation of the RAAS and sympathetic nervous system. However, this increased activity paradoxically further decreases the GFR, leading to impaired renal function. Thus, a reciprocal relationship of structural and functional damage begins between the heart and kidney and this relationship turns into a chronic vicious cycle over time (Jois and Mebazaa, 2012).

Materials and Methods

The material of this study consisted of a total of 25 cats of different breeds, age, sex and body weight. On the basis of cardiological examination, they were classified according to the ACVIM grading system (Keene et al., 2019) and evaluated in two different groups. In this context: Control group (n=10) and test group (n=15). The test group consisted of cats with cardiomyopathy that was detectable and diagnosable at the symptomatic level based on examination results, which were brought to the Internal Medicine Clinic of the Animal Hospital at Kırıkkale University Faculty of Veterinary Medicine by their owners, who assumed responsibility for all diagnostic and treatment costs. The control group consisted of healthy cats.

This study was evaluated by Kırıkkale University Animal Experiments Local Ethics Committee (Number no: E-60821397-605-341747) within the scope of "Clinical applications for diagnosis and treatment purposes" specified in the "k" clause of Article 8 of the Regulation on Working Procedures and Principles of Animal Experiments Ethics

Committees.

The selection of the cases included in the study was based on ACVIM criteria (Keene ., 2019). Classes A and B were asymptomatic and classes C and D were symptomatic patient groups. General examination, blood ProBNP, SDMA and serum biochemical analyses, X-ray, ECG and echocardiographic examinations, hypertrophic cardiomyopathy (HCM, n=10), hypertrophic obstructive cardiomyopathy (HOCM, n=4) and unclassifiable cardiomyopathy (UCM, n=1) cases with renal involvement were included in the study.

The control group consisted of healthy cats without any complaints who were brought to Kırıkkale University Animal Hospital. Cats were examined clinically, radiologically, electrocardiographically and echocardiographically under optimum conditions and the cases which were determined to be healthy based on complete blood count, serum biochemical results and ProBNP levels (K.Ü. Veterinary Faculty Animal Hospital) were included in the study. All vital signs of the cats in the test and control groups were evaluated, standard clinical diagnosis and treatment procedures were applied and the data were recorded. Thoracic radiography was performed after clinical examination of the patients, and abnormal auscultation and inspection findings diagnosed from the anamnesis were supported by radiological imaging. Two poses of thorax radiographs, lateral and ventrosorsal, were taken from each patient and the radiographic morphology of the heart, lungs and thoracic vessels were evaluated together with vertebral heart score (VHS) measurements.

ECG examination was performed with bipolar and unipolar limb leads (Carewell 1103G Veterinary ECG device) in the right parasternal recumbent position in a quiet, calm, minimal stress environment without the use of sedative agents using a properly arranged ECG and echocardiography unit in the test group cats. Cardiac rhythm analyses and ECG wave and interval measurements were performed from the second lead with a standard calibration (10 mm/mV, 50 mm/sec, DII) (Wendy, 2011).

Echocardiographic examination was performed without sedation. Cardiac measurements were obtained by transthoracic echocardiography; standard methods (two-dimensional (2-D), M-mode and colour Doppler) and imaging techniques (right parasternal short and long axis, left apical 4-5 cavity windows) were used for this purpose. In accordance with the echocardiographic examination technique, the right parasternal short-axis papillary muscle level was imaged and the LV, papillary muscles, IVS and RV

structures were examined by 2-D echocardiography from the right parasternal short-axis papillary muscle level and their geometric and functional measurements were noted. In this image, LV size, IVS and LVFW were measured as cardiac geometry in systole and diastole, while functionally LV volume, %FS and %EF values at end-systole and end-diastole were calculated automatically by the device (Boon, 2011). While in this window, the aortic (Ao) level was obtained by slightly rotating the probe in the right direction and raising the wrist slightly. During this time, care was taken to ensure that the aorta was clearly visible as a central, circular structure with three leaflets (semilunar valves); the left atrium (LA) was visible at the 6-7 o'clock position, and the main pulmonary artery (MPA) was visible at the 3 o'clock position. With this image, the diameters of the Ao, LA, and MPA were measured, and the LA/Ao ratio was calculated. Dimensional and, when necessary, color Doppler measurements were performed.

Following the short axis view in the right parasternal position, the long axis 4-5 cavity view was obtained by rotating the probe clockwise to the left. Diastolic LA and RA diameters and systolic LVOT and Ao root diameters were obtained, respectively. During five-chamber imaging, M-mode measurements were performed by placing the ultrasound cursor at a 90-degree angle to the ventricular septum and tangentially passing through the apex of the mitral valve opening. Before the right parasternal position was changed, colour Doppler measurements of the MPA were performed by returning to the Ao level short axis image and PA maximum velocity and ejection times were obtained by first CW and then PW measurements (Boon, 2011).

Left apical 4-5 cavity images were preferred to evaluate the right and left atria and ventricles in terms of diameter and volume and colour Doppler characteristics of MV, TV and Ao flows were obtained in this window. Serial measurements were performed in CW and PW mode by positioning the ultrasound cursor at the peaks of MV and TV, respectively; MV and TV E/A ratios were automatically calculated by the device after scanning the flows. In the position where the left ventricular aortic outflow became clearly visible, the Ao flow characteristic was obtained with an ultrasound cursor placed parallel to the flow, and the maximum velocity and ejection times were measured from the right triangular formation below the baseline line (Boon, 2011).

These analyses consisted of serum biochemical measurements (ProBNP, Na-K-Cl, BUN, Crea) together with a complete blood count (Nx600

Biochemistry, Fujifilm and Idexx catalyst one). Blood samples (Bc60 Haemogram, Mindray) were collected from the cephalic veins of the cats using the appropriate technique in tubes with anticoagulant (EDTA) for haemogram and without anticoagulant for serum biochemical measurements.

Serum samples were analysed for electrolytes (Na-K-Cl) and markers of renal damage (blood urea nitrogen and creatinine) (Nx600 Biochemistry). SDMA was also analysed in all patients (Idexx catalyst one). Serum cardiac ProBNP was measured (Vcheck V200) and evaluated with the appropriate kit.

In the statistical analyses of the data obtained, descriptive statistics were performed and the data were tabulated as mean and standard deviation. The homogeneity of the data was evaluated using Shapiro- Wilk analysis and their normal distribution was checked. It was determined that the data that did not show normal distribution did not provide normal distribution although the transformation process was used according to Logbase. In this context, Mann-Whitney U test, which is a non-parametric test, was used for intergroup comparisons. In addition, Pearson correlation was used to evaluate the correlations between the data obtained from echocardiographic measurements and haematological and other biochemical measurements. In all analyses, P values less than 0.05 were considered statistically significant and SPSS 29.0 (IBM, USA) was used in all analyses.

Results

Results; ACVIM heart failure classification (class B2, C and D) was established and renal effects were evaluated with parameters. Cats without a diagnosis of heart failure (class A) formed the control group (n:10) and renal effects were evaluated. A total of 25 cats of different breeds, age and sex were used in this study.

The cats included in the study were reported to have complaints of heart disease for at least 2 months (maximum 2 years). These complaints were primarily noted as exercise intolerance (12/15), lethargy (3/15), respiratory distress (5/15), vomiting (6/15), polyuria-polydipsia (4/15), diarrhoea (1/15), decreased appetite (9/15), systolic murmurs (6/15) and weight loss (7/15). In physical examinations, parameters such as pallor of the mucous membranes and fullness of the jugular vein, which were recognisable on inspection due to cardiac pathologies, were added to the patient records. As a result of the analyses, although body weight averages were found to be similar between both groups, significant differences were observed in the minimum and maximum value ranges. When the

mean age data were compared, the mean age of the cats in the test group (4.06 ± 4.01 years) was significantly lower than that of the control group (6.78 ± 4.15 years for the control group and 8.07 years for the maximum value) and this difference was found to be statistically significant ($p < 0.05$). Within the scope of physical evaluation, vital signs such as body temperature (T°), heart rate (P), respiratory rate (R) and capillary refill time (CRT) were also recorded in both groups of cats. There was a significant difference between the T values between the groups; P and R measurements in the test group were found to be statistically significantly higher than those in the control group ($p < 0.05$) (Table 1). When the vertebral heart score (VHS) was examined radiologically, a significant increase was found in the test group compared to the control group (7.75 ± 0.35 and 9.51 ± 0.70 ; $p < 0.001$) (Table 2).

Table 1. Signalement and some clinical findings of the control and test groups; mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
W (kg)	Control	5.8 ± 1.60	3.8 - 8.5	0,019
	Test	4.22 ± 0.97	2.3 - 5.3	
Age (year)	Control	6.78 ± 4.15	2.4 - 17	0,031
	Test	4.06 ± 4.01	0.6 - 17	
T ($^\circ$ C)	Control	38.48 ± 0.36	38 - 39.1	0,016
	Test	37.48 ± 1.56	32.4 - 39	
P (min)	Control	146.40 ± 20.39	126 - 192	<0,001
	Test	207.73 ± 22.06	162 - 234	
R (min)	Control	26.00 ± 3.65	22 - 32	<0,001
	Test	40.93 ± 9.37	26 - 56	
CRT (sec)	Control	1.84 ± 0.25	1.4 - 2.2	0,103
	Test	1.62 ± 0.38	1 - 2.5	

W: Live weight T: Body temperature, P: Pulse, R: Respiration, CRT: Capillary refill time

Table 2. Vertebral heart scores (VHS) of control and test group cases; mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
VHS	Control	7.75 ± 0.35	7.2 - 8.4	<0,001
	Test	9.51 ± 0.70	8.1 - 10.6	

In ECG examination, respiratory sinus arrhythmia was detected in some (3/10) of the control group cats in which rhythm analysis was performed. In the test group, sinus tachyarrhythmia (5/15) and atrial fibrillation (4/15) as well as atrial (3/15) and ventricular premature complexes (3/15) were detected. Along with this rhythm evaluation, morphological evaluations were made and the duration and voltages of ECG waves were examined. Accordingly; left atrial dilatation (P-mitrale, 2/15), right atrial dilatation

(P-pulmonale, 3/15), left ventricular enlargement (5/15), small QRS complex formation (2/15), ST depression (2/15), pointed T wave (4/15) and left or right axis deviation (5/10) were the primary findings. In echocardiographic evaluations performed in patients with a diagnosis of heart failure, it was frequently noted that the left atrium was markedly enlarged, mitral valve prolapse findings were observed, and insufficiency flows were observed in the mitral and tricuspid valves. In addition, structural changes such as narrowing of the left ventricular outflow tract (LVOT), thickening of the interventricular septum (IVS) and left ventricular posterior wall (LVPW), as well as significant increases in the LA/Ao ratio were found. According to these findings, the IVSd value of the test group (0.60 ± 0.07 cm) was significantly increased compared to the mean in the control group (0.48 ± 0.09 cm) and this difference was found to be statistically significant ($p < 0.001$). Although there was no statistical difference between the groups in systole and diastole phases of IVS measurements, LVDd and LVDs diameters were significantly higher in the test group, respectively ($p < 0.01$ and $p < 0.05$). Fractional shortening (FS) rate, which is a functional evaluation parameter, was significantly lower in the test group ($46.21\% \pm 12.46\%$) compared to the control group ($53.90\% \pm 9.37\%$) ($p < 0.01$) (Table 3).

Table 3. M-mode measurements taken from the right parasternal short axis papillary muscle level in the control and test groups; mean $\pm$ standard error (cm), minimum-maximum, and p-value.

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
IVSd	Control	0.48 ± 0.09	0,29 - 0,6	0,001
	Test	0.60 ± 0.07	0,5 - 0,79	
LVDd	Control	1.49 ± 0.24	1,01 - 1,78	0,238
	Test	1.34 ± 0.27	0,96 - 1,74	
LVPWd	Control	0.55 ± 0.09	0,36 - 0,7	0,002
	Test	0.67 ± 0.07	0,52 - 0,83	
IVSs	Control	0.73 ± 0.12	0,54 - 0,94	0,978
	Test	0.74 ± 0.12	0,52 - 1,03	
LVDs	Control	0.68 ± 0.14	0,44 - 0,87	0,683
	Test	0.73 ± 0.25	0,31 - 1,2	

LVPWs	Control	0.80 ± 0.12	0,62 - 1,02	0,605
	Test	0.82 ± 0.09	0,67 - 0,99	
FS %	Control	53.90 ± 9.37	34,69 - 64,77	0,091
	Test	46.21 ± 12.46	25 - 67,44	
EF %	Control	86.70 ± 7.73	68,96 - 94,17	0,129
	Test	79.00 ± 11.99	53,45 - 95,72	

RVDd - Right ventricular diastolic diameter; interventricular septum diastolic (IVSd) and systolic diameter (IVSs); left ventricular diastolic (LVDd) and systolic diameter (LVSd); left ventricular free wall diastolic (LVFWd) and systolic diameter (LVFWs); FS - fractional shortening; and EF - ejection fraction

No significant difference was found between the groups in terms of Ao and MPA diameters. However, LA diameter was significantly higher in the test group (1.57 ± 0.32 cm) than in the control group (1.20 ± 0.21 cm). In parallel, LA/Ao ratio was significantly increased in the test group (1.96 ± 0.36) and this difference was statistically significant ($p < 0.01$) (Table 4).

Table 4. Right parasternal short-axis aortic diameter measurements in the control and test groups. Ao: Aorta, LA: Left atrium, and MPA: Main pulmonary artery (mean $\pm$ standard error), cm – centimeters, minimum-maximum, and p-value.

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
Ao	Control	8.14 ± 23.13	0,71 - 74	0,177
	Test	0.79 ± 0.10	0,61 - 1,04	
LA	Control	1.20 ± 0.21	0,83 - 1,57	0,003
	Test	1.57 ± 0.32	0,99 - 2,29	
MPA	Control	0.88 ± 0.08	0,81 - 1,05	0,129
	Test	0.81 ± 0.10	0,5 - 0,94	
LA/Ao	Control	1.45 ± 0.32	1,09 - 2,22	0,002
	Test	1.96 ± 0.36	1,47 - 2,5	

The maximal blood flow velocities (velocities) in the aorta and pulmonary arteries and flow profiles through the mitral (MV) and tricuspid (TV) valves were examined by colour Doppler echocardiography and the E/A ratios of MV and TV valves did not show a statistically significant difference in both groups. While the TV-E/A ratio was between 1.07 and 1.59 in the control group, this ratio was

between 0.93 and 3.1 in the test group (Table 5).

Table 5. Doppler measurements (mean $\pm$ standard error), minimum-maximum, and p-value of pulmonary artery (PA), aorta (Ao), mitral valve (MV), and tricuspid valve (TV) in the control and test groups.

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	P
PA Vmax, (cm/sn)	Control	78,11 $\pm$ 12,73	54,79 – 94,28	0,129
	Test	104,25 $\pm$ 45,63	36,34 - 207,64	
Ao Vmax mmHg	Control	96,81 $\pm$ 20,81	74,88 – 136,99	0,129
	Test	166,03 $\pm$ 100,90	61,72 - 378,37	
MV E/A	Control	1,23 $\pm$ 0,23	1,03 - 1,74	0,978
	Test	1,27 $\pm$ 0,45	0,79 - 2,77	
TV E/A	Control	1,25 $\pm$ 0,18	1,07 – 1,59	0,892
	Test	1,42 $\pm$ 0,68	0,93 - 3,1	

According to the leucogram data, no statistically significant difference was found between the test and control groups in terms of total leucocyte (WBC) counts and differential leucocyte distribution (especially neutrophil and lymphocyte ratios) (Table 6).

Table 6. Leukogram values ($\times 10^3$ / L) of control and test group cases; mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
WBC $\times 10^3$ /mL	Control	8,58 $\pm$ 3,96	4,13 - 16,6	0,196
	Test	9,88 $\pm$ 2,44	5,99 - 13,78	
Lymphocyte $\times 10^3$ /mL	Control	2,60 $\pm$ 1,70	0,74 - 6,62	0,461
	Test	2,02 $\pm$ 1,31	0,38 - 4,88	
Neutrophil $\times 10^3$ /mL	Control	5,49 $\pm$ 3,14	1,54 - 12,11	0,196
	Test	7,29 $\pm$ 3,18	2,30 - 12,75	
Monocyte $\times 10^3$ /mL	Control	0,12 $\pm$ 0,12	0,02 - 0,43	0,338
	Test	0,17 $\pm$ 0,13	0,04 - 0,47	
Eosinophil $\times 10^3$ /mL	Control	0,35 $\pm$ 0,23	0 - 0,75	0,723
	Test	0,37 $\pm$ 0,38	0 - 1,16	
Basophil $\times 10^3$ /mL	Control	0,01 $\pm$ 1,82	0,01 - 0,01	0,807
	Test	0,01 $\pm$ 0,01	0 - 0,06	

However, remarkable elevations were detected in some individual samples of the test group. In particular, WBC and neutrophil counts in the test group reached 13.78/ μ L and 12.75/ μ L, respectively, which may indicate possible inflammatory or stress responses in these animals. In the erythrogram values, as in the leucogram, no statistically significant differences were detected in the averages of both groups. The erythrocyte (RBC) count, haemoglobin,

haematocrit (Hct) and erythrocyte index values (MCV, MCH, MCHC and RDW) (Table 7) of the control group were within normal limits. Platelet count (PLT) and indices (PCT, MPV and PDW) were recorded as thrombogram values (Table 8). When the mean values were analysed, no statistically significant differences were found in the mean values of both groups as in erythrogram and leucogram.

Table 7. Erythrogram values of control and test group cases; mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
RBC $\times 10^6$ (mL)	Control	9,24 $\pm$ 1,50	6,05 - 11,66	0,567
	Test	8,26 $\pm$ 2,46	3,69 - 11,35	
HCT (%)	Control	39,38 $\pm$ 6,02	27,4 - 48	0,605
	Test	34,97 $\pm$ 10,81	17,6 - 47,8	
HGB (gr/dL)	Control	14,01 $\pm$ 2,02	9,9 - 16,7	0,428
	Test	12,44 $\pm$ 3,88	5,9 - 17	
MCV (gr/dL)	Control	42,80 $\pm$ 3,29	37,2 - 47,3	0,683
	Test	42,31 $\pm$ 2,92	37,6 - 47,8	
MCH (gr/dL)	Control	15,24 $\pm$ 1,13	13,8 - 17,1	0,849
	Test	15,01 $\pm$ 1,06	13,1 - 16,7	
MCHC (gr/dL)	Control	356,40 $\pm$ 10,88	336 - 372	0,531
	Test	333,90 $\pm$ 83,11	35,5 - 372	
RDW (%)	Control	17,57 $\pm$ 0,83	16,3 - 19	0,237
	Test	18,42 $\pm$ 1,19	16,4 - 20,4	

RBC – red blood cell count; Hgb – hemoglobin; Hct – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; and RDW – red blood cell distribution width (shape and volume)

Table 8. Thrombogram values of control and test group cases; mean $\pm$ standard error, minimum-maximum, and P value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
Platelet $\times 10^6$ (mL)	Control	210,40 $\pm$ 96,58	62 - 348	0,723
	Test	207,40 $\pm$ 89,67	68 - 458	
PCT (%)	Control	2,39 $\pm$ 0,88	0,91 - 3,41	0,807
	Test	2,43 $\pm$ 1,18	0,28 - 5,54	
MPV fL	Control	12,02 $\pm$ 1,95	9,2 - 14,8	0,428
	Test	12,80 $\pm$ 1,47	10,1 - 16,4	
PDW (%)	Control	15,22 $\pm$ 0,91	13,9 - 17,1	0,397
	Test	14,99 $\pm$ 0,66	13,9 - 16,4	

Serum N-terminal pro B-type natriuretic peptide (NT-proBNP) was studied as a cardiac biomarker;

the mean value of the test group (1267.46 ± 310.84 pmol/L; $p < 0.001$) was found to be higher than the control (70.63 ± 16.47 pmol/L) (Table 9).

Table 9. Serum NT-proBNP values of control and test group cases, mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
NT-ProBNP Pmol/L	Control	70,63 $\pm$ 16,47	50 - 98	<0,001
	Test	1267,46 $\pm$ 310,84	543 - 1500	

In terms of renal function, BUN and CREA values were significantly higher in the test group (138.2 mg/dL and 4.96 mg/dL, respectively) compared to the controls (30.3 mg/dL and 1.5 mg/dL). BUN and CREA values in the test group (63.44 ± 28.77 mg/dL, 2.51 ± 0.98 mg/dL) were significantly higher than the control group (23.68 ± 5.39 mg/dL, 1.22 ± 0.28 mg/dL). BUN and CREA data in the test group were higher ($P < 0.01$) than those found in the control group. Other parameters analysed were electrolyte panel (sodium (NA), potassium (K), chlorine (CL)) (Table 10) and symmetric dimethylarginine (SDMA) (Table 11). Although there was no statistical significance for serum Sodium (NA) and Chlorine (CL) values between the control and test groups, the values were mostly within the reference ranges reported for cats. Potassium (K) and SDMA values in the test group (4.60 ± 0.65 mmol/L, 19.53 ± 60.03 mg/dL) were higher than the control group (4.03 ± 0.49 mmol/L, 11.40 ± 1.50 mg/dL). K and SDMA data were higher ($P < 0.01$) in the test group compared to the control group. Although there was no statistical significance for serum Sodium (NA) and Chlorine (CL) values between the control and test groups, the values were mostly within the reference ranges reported for cats. Potassium (K) and SDMA values in the test group (4.60 ± 0.65 mmol/L, 19.53 ± 60.03 mg/dL) were higher than the control group (4.03 ± 0.49 mmol/L, 11.40 ± 1.50 mg/dL). K and SDMA data were higher ($p < 0.01$) in the test group compared to the control group.

Table 10. Serum biochemical values of control and test group cases, mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
CREA (mg/dL)	Control	1,22 $\pm$ 0,28	0,73 - 1,5	<0,001
	Test	2,51 $\pm$ 0,98	1,73 - 4,96	

BUN (mg/dL)	Control	23,68 $\pm$ 5,39	17 - 30,3	<0,001
	Test	63,44 $\pm$ 28,77	33,2 - 138,2	
Na (mEq/L)	Control	147,50 $\pm$ 4,74	139 - 154	0,177
	Test	149,73 $\pm$ 8,67	132 - 162	
K (mEq/L)	Control	4,03 $\pm$ 0,49	3,5 - 5,1	0,016
	Test	4,60 $\pm$ 0,65	2,8 - 5,4	
Cl (mEq/L)	Control	111,70 $\pm$ 4,19	106 - 118	0,428
	Test	108,07 $\pm$ 8,93	81 - 118	

Table 11. Serum SDMA values of control and test group cases, mean $\pm$ standard error, minimum-maximum, and p-value

Parameter	Groups	Mean $\pm$ Standard Deviation	Min-Max	p
SDMA (mg/dL)	Control	11,40 $\pm$ 1,50	9 - 14	<0,001
	Test	19,53 $\pm$ 60,03	14 - 38	

Discussion

In this study, renal dysfunction due to cardiomyopathy in cats was investigated. The similarity of the anamnesis and physical examination findings obtained for the test group of this study material to previous studies (Schober et al., 2010) showed once again the importance of the diagnostic process and clinical findings related to heart failure in cats.

A weak negative correlation was found between serum creatinine level and body weight (kg) ($r = -0.500$, $p = 0.058$). This finding is within the limit of statistical significance. In cats with chronic renal failure due to cardiomyopathy, weight loss is usually associated with protein calorie malnutrition, muscle wasting and chronic catabolic processes (Ross, 2009). A low level negative correlation was found between BUN and body temperature (T) ($r = -0.055$, $p = 0.846$). Although this finding is far from statistical significance, it is important in terms of the tendency towards hypothermia that may be observed in some cases of advanced azotemia. Hypothermia may occur in cases of severe dehydration, hypoperfusion and metabolic dysfunction (DiBartola, 2012). A significant and negative correlation was found between creatinine and RBC ($r = -0.568$, $p = 0.027$). This suggests that there may be a tendency for a decrease in respiratory rate as renal function deteriorates. As chronic renal failure progresses, metabolic acidosis may develop, which may initially increase respiration, but in later

stages may suppress respiratory rate with systemic effects such as fatigue, hypoventilation and even uremic encephalopathy (DiBartola, 2012). Although a negative correlation was found between NT-proBNP and pulse rate (PR), it was not statistically significant ($r = -0.208$, $p = 0.456$). As renal function decreases, weakness and fatigue, reluctance to walk (Börkür et al., 2000), respiratory distress and dyspnoea are observed in chronic heart failure (Ware, 2011). A weak positive correlation was found between NT-proBNP and VHS ($r = 0.091$, $p = 0.746$). This finding was not statistically significant. NT-proBNP levels increase as ventricular wall tension increases, which is usually associated with cardiomegaly (Ware, 2011). A low positive correlation was found between SDMA and VHS ($r = 0.252$, $p = 0.365$). This correlation supports that renal dysfunction develops as a result of impaired renal perfusion as cardiac dysfunction progresses (Ronco et al., 2008). During ECG examination of the patients, rhythm analyses were performed; morphologically detected left atrial enlargement (P-mitrale), right atrial enlargement (P-pulmonale) and left ventricular enlargement were found to increase in R amplitude, prolongation in QRS duration and reflection on deep Q waves and became evident in patients with cardiomyopathy. Since the common clinical finding in HCM is diastolic dysfunction and the mitral valve is open in diastole, the pressure present in the LV in diastole is also present in the left atrium (LA). As a result, an increase in LV diastolic pressure causes an increase in LA pressure, and an increase in LA pressure causes LA dilatation (Kittleson and Cote, 2021). In this study, geometric and functional variations were recorded in cardiomyopathies and LA diameter was often measured higher in the test group (1.57 ± 0.32) than in the control group (1.20 ± 0.21). A positive and significant correlation was found between NT-proBNP and left atrial diameter (LA) ($r = 0.579$, $p = 0.024$). This finding supports the information described in the literature that NT-proBNP is directly related to left atrial volume and increased intracardiac pressure. A strong positive correlation was also found between NT-proBNP and LVPW ($r = 0.832$, $p = 0.060$). This indicates that left ventricular wall hypertrophy, which is characteristic of HCM, increases NT-proBNP levels (Saponaro et al., 2022). In this study, the fact that the majority of LVDd, LVDs and LA/Ao values were higher than control and reference values (Boon, 2011) in cats with cardiomyopathy revealed the geometric remodelling in the left heart. SDMA was positively correlated with left ventricular diameter (LVDd) ($r = 0.404$, $p = 0.404$). This correlation suggests the effect of decreased perfusion due to cardiomyopathy on renal function. NT-proBNP was unexpectedly negatively correlated with LA/Ao ratio ($r = -0.579$, $p = 0.024$). This result deviates from the positive

correlation trend reported in the classical literature and may be explained by various physiological and methodological reasons. This is because NT-proBNP is associated not only with LA size but also with left ventricular hypertrophy, diastolic dysfunction and systemic hypertension (Saponaro et al., 2022). Accordingly, although the LA/Ao ratio is low, NT-proBNP may increase. In our study, %EF value showed variable values in the test group (79.00 ± 11.99) compared to the control group (86.70 ± 7.73) and %FS value in the test group (46.21 ± 12.46) compared to the control group (53.90 ± 9.37). The significant increase in MV-E/A and TV-E/A ratios in the test group compared to the control group was an indicator of diastolic dysfunction in cats (Smith et al., 2016). No significant correlation and significant statistical results were found between AoVmax values and biochemical markers. A significant negative correlation was found between NT-proBNP and PAVmax ($r = -0.551$, $p = 0.033$). This finding suggests that right heart volume increases with the progression of heart failure and pulmonary artery flow velocity decreases due to increased pulmonary vascular resistance. Therefore, it suggests that left heart failure may affect the right heart secondarily and this may cause an increase in NT-proBNP levels (Reinero et al., 2020). In the test material of this study, heart failure was not classified according to the etiological causes; HCM, HOCM and UCM cases, which are frequently seen in cats, were mentioned (Kittleson and Cote, 2021). We believe that this study may form an infrastructure for comprehensive studies in which the follow-up and treatment process will be planned as a different group from chronic (congestive) heart failure, which is the common and final stage of the aforementioned diseases, as well as the mentioned cardiomyopathies.

The statistically higher WBC and neutrophil counts in the test group patients compared to the control group patients suggested the presence of stress leukogram and SIRS (systemic inflammatory response syndrome). Stress leukogram is characterised by neutrophilic leukocytosis with lymphopenia, eosinopenia, monocytosis and/or basophilia (Willard, and Tvedten, 2012). A negative correlation was found between ProBNP and WBC value ($r = -0.507$, $p = 0.054$), but this finding has statistically limited significance. This may be explained by immunosuppression or chronic inflammation process. A significant positive correlation was found between SDMA and monocyte ratio ($r = 0.529$, $p = 0.043$). This finding suggests that the increase in monocytes, especially in early renal dysfunction, is a haematological reflection of systemic inflammation. The negative correlation between NT-proBNP and basophil ratio is quite strong ($r = -0.708$, $p = 0.003$), which is in line with the immune modulation

seen in heart and kidney failure reported in the literature (Weiss, 2020; Reinero et al., 2020). HGB ($r = -0.513$, $p = 0.051$) and HCT ($r = -0.506$, $p = 0.054$) show a moderate negative correlation. This indicates that haematological structure is suppressed as renal function deteriorates. Similarly, there is a significant negative correlation between SDMA and HGB ($r = -0.540$, $p = 0.038$) and HCT ($r = -0.534$, $p = 0.040$). These findings suggest that SDMA may be a useful biomarker in the early detection of renal involvement as well as in the evaluation of the effect of renal anaemia. A significant and negative correlation was found between SDMA and MCH ($r = -0.614$, $p = 0.015$) and MCHC ($r = -0.521$, $p = 0.047$) as well as between creatinine and MCH ($r = -0.542$, $p = 0.037$), but these did not reach statistical significance. A strong negative correlation was found between creatinine and MPV ($r = -0.621$, $p = 0.013$) and between BUN and MPV ($r = -0.873$, $p < 0.001$), as well as a similarly significant negative correlation between SDMA and MPV ($r = -0.733$, $p = 0.002$). These findings suggest that platelets become structurally smaller or undergo functional suppression as renal function deteriorates. A significant positive correlation ($r = 0.597$, $p = 0.019$) was found between SDMA and platelet count (PLT). This indicates that PLT count increases as SDMA level increases, but evaluation with platelet function tests and acute phase protein levels is necessary to clarify whether it is reactive thrombocytosis due to inflammation (Reinero et al., 2020). The high correlation between BUN and CREA values ($r = 0.738$, $p = 0.002$) is an indication of parallel deterioration in renal function. A strong and significant positive correlation was found between SDMA and creatinine ($r = 0.750$, $p = 0.001$). The negative correlation between NT-proBNP and SDMA ($r = -0.357$, $p = 0.191$) is in accordance with the model of chronic kidney disease secondary to chronic heart failure described in the literature (Chuasuwat and Kellum, 2012). Negative correlation trends were observed between creatinine and CL ($r = -0.206$, $p = 0.443$) and SDMA and CL ($r = -0.195$, $p = 0.486$). This suggests that hypochloremia may develop as renal function deteriorates and that this may be particularly associated with metabolic alkalosis or renal tubular dysfunction (DiBartola, 2012). A weak positive correlation was found between creatinine and K ($r = 0.173$, $p = 0.173$), suggesting that the tendency for hyperkalaemia may develop in parallel with progressive renal dysfunction. A moderate negative correlation was found between NT-proBNP and K levels ($r = -0.499$, $p = 0.058$). Increased aldosterone level as a result of RAAS activation causes sodium and water retention in the kidneys and increases the excretion of potassium from the distal tubule. This increases the risk of hypokalaemia, especially in

patients with chronic heart failure (DiBartola, 2012). In this study, it can be said that neurohormonal haemostasis (SNS and RAAS activations) in response to cardiomyopathy in cats (Urso et al., 2015) was effective in keeping serum electrolyte levels in the test group partially within acceptable limits.

Conclusion

The complex physiopathological relationship between heart and kidney is not only limited to primary systemic involvement, but also triggers mutual loss of function with feedback effect between the organs. In this context, early evaluation of renal function, especially in cases of heart failure due to cardiomyopathy, has become critical in terms of recognition and prognosis. In this study; the findings revealed that renal involvement secondary to cardiomyopathy can be identified with multifaceted laboratory and imaging data. Since renal involvement may often be subclinical in cats with cardiac disease, it is recommended that regular screening and check-up protocols be established and disseminated in veterinary practice. In order to monitor cardiac and renal functions together, screening of new generation biomarkers such as NT-proBNP and SDMA as primary sources and their combined use with classical diagnostic tools (haemogram, biochemistry, imaging) should be integrated into standard protocols. Regular monitoring of haematological changes (nonregenerative anaemia, platelet dysfunction) in patients with cardiomyopathy and associated progressive renal failure and chronic heart failure will ensure early recognition of the multisystemic effects of the disease. Haemodynamic findings support the catabolic effects of heart failure on circulation and renal perfusion. In order to elucidate the exact physiopathology of renal involvement in cats, long-term follow-up studies and repeated biomarker analyses at tissue level will be useful.

Supporting the correlations observed in this study with further studies with larger case groups and samples will increase the generalisability of the data obtained.

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Authors' Contributions:

DK and BBY were actively involved in all stages of the study, from the initial conception to the design and implementation. They contributed to data collection, analysis, and interpretation, and played a significant role in drafting and writing the manuscript. All authors have read and approved the final version of the manuscript.

Ethical approval:

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (Approval No: E-60821397-605-341747).

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Haemotropic Mycoplasmosis in a Cat

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Abstract

Feline hemotropic mycoplasmosis is a rickettsial disease that infects red blood cells and is caused by *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum*, and *Candidatus Mycoplasma turicensis*. An 8-month-old female domestic cat that had anorexia and weakness is the subject of this case study. Tachycardia, increased rectal body temperature, and icteric mucous membranes were found in clinical examination. Serum biochemistry evaluation revealed elevated activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzyme activities, as well as increased total and direct bilirubin concentrations. Hematological evaluation showed anemia and leukocytosis characterized with lymphocytosis, monocytosis and neutropenia. Blood smear examination confirmed the presence of *Mycoplasma haemofelis*. After receiving oxytetracycline and methylprednisolone for 10 days, the clinical findings of the cat significantly improved. Feline hemotropic mycoplasmosis should be included in the differential diagnosis for cats presented with icterus, increased rectal body temperature and anemia.

Keywords: Anemia, cat, hemotropic mycoplasma

INTRODUCTION

Hemotropic mycoplasmas are gram-negative, cell wall-deficient bacteria that adhere to the surface of red blood cells, leading to hemolytic anemia. In cats, three species have been identified: *Mycoplasma haemofelis*, *Candidatus Mycoplasma turicensis*, and *Candidatus Mycoplasma haemominutum*. *M. haemofelis* is recognized as most prevalent and pathogenic mycoplasma species infecting cats. These microorganisms are primarily transmitted through blood-feeding arthropods such as fleas, lice, ticks, and mosquitoes. Additionally, transmission can occur via intrauterine and galactogenic routes, bite wounds from cat fights, or contaminated blood transfusions (Tasker et al., 2018)

The severity of the clinical signs associated with the disease may vary depending on the infecting *Mycoplasma species*, the presence of concurrent infections

(e.g. retrovirus infections) and the stage of infection (Tasker, 2010). In cats suffered from acute hemotropic mycoplasmosis caused by *Mycoplasma haemofelis* present clinical signs including dullness, inappetence, weight loss, lymphadenopathy, anemia, and increased rectal body temperature. Icterus is uncommon and typically occurs in cases of severe acute hemolysis. Diagnosis is based on clinical and hematological findings, microscopic examination of a peripheral blood smear and observation of single, double or sometimes arranged round bodies on the erythrocyte surface, or polymerase chain reaction (Ameldev and Tresamol, 2017; Barker, 2019).

Although the treatment with tetracycline and quinolone group antibiotics have been reported to reduce the number of organisms in the blood, there is no study to show that the organism can be completely eliminated from the blood with these

group antibiotics. The use of corticosteroids is also recommended to suppress immune-mediated hemolytic anemia associated with hemotropic mycoplasma infection in cats (Tasker et al. 2018).

The aim of this case report was to present the clinical, hematological, and biochemical findings in a cat diagnosed with hemotropic mycoplasmosis.

Case description

The material of the case was an 8-month-old female cat that was presented to the Veterinary Teaching Hospital of Kastamonu University Faculty of Veterinary Medicine with the complaints of anorexia and weakness. The owner reported that the cat was kept in the garden, had anorexia and weakness for the last two days, and had not received any preventive vaccinations.

Clinical examination revealed icteric mucous membranes (Figure 1).

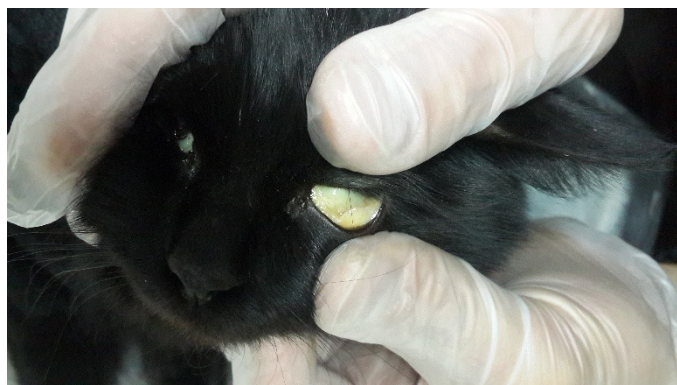


Figure 1. An icteric appearance of the conjunctiva before treatment

The rectal body temperature was recorded as 39.4°C, and the heart rate was 160 beats per minute. Blood samples were collected from the vena cephalica antebrachii into EDTA containing and plain tubes for hematological and biochemical analyses respectively. A peripheral blood smear was also prepared and stained with Giemsa. Hematological examinations were performed using an automated veterinary analyser (iVet5, Norma Diagnostika, Budapest, Hungary). Serum biochemistry analysis was conducted using a dry chemistry analyzer (FujiDri-Chem NX500i, Fujifilm Corporation, Tokyo, Japan). The presence of Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) antigens and antibodies were qualitatively evaluated using an immunochromatographic lateral flow test kit (FeLV-FIV Diagnostic Kit, Uranovet, Spain).

Haematological examination revealed severe leukocytosis ($30.1 \times 10^3/\mu\text{L}$), characterised by

neutropenia, lymphocytosis and monocytosis, macrocytic anaemia and thrombocytopenia (Table 1). Biochemical examination showed increased activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), along with elevated total and direct bilirubin levels (Table 1).

Table 1. Hematology and serum biochemistry analysis results

Parameters	Results	Reference Values
WBC ($\times 10^3/\mu\text{L}$)	30.1	5.1-16.2
Lymphocyte (%)	64	9-56
Monocyte (%)	20	0-6
Neutrophil (%)	15	27-82
RBC ($\times 10^6/\mu\text{L}$)	2.32	6.9-10.1
Hemoglobin (g/dL)	5.2	10.9-15.7
HCT (%)	13.8	31-48
MCV (fL)	59.7	40-52
MCHC (g/dL)	37.6	32-35
ALT (U/L)	511	28-109
AST (U/L)	394	17-48
Creatinine (mg/dL)	0.5	0.8-2.1
Total Bilirubin (mg/dL)	16.4	0-0.1
Direct Bilirubin (mg/dL)	7.4	0-0

Microscopic examination of the blood smear showed the structures on the surface of the erythrocytes consistent with *M. haemofelis* (Figure 3). FeLV antigen and FIV antibody presence were also not detected in this cat. Based on the results obtained from clinical and laboratory examinations, hemotropic mycoplasmosis was diagnosed. The treatment was initiated including oxytetracycline (Pan-Terramycin 3% fl., Zoetis) at a dose of 10 mg/kg once daily, and methylprednisolone (Deltacortril tablet 5 mg, Pfizer) at a dose of 1 mg/kg once daily for a period of 10 days. After the treatment period, appetite, mobility, and mucous membrane color returned to normal (Figure 2).



Figure 2. Appearance of the conjunctiva after treatment

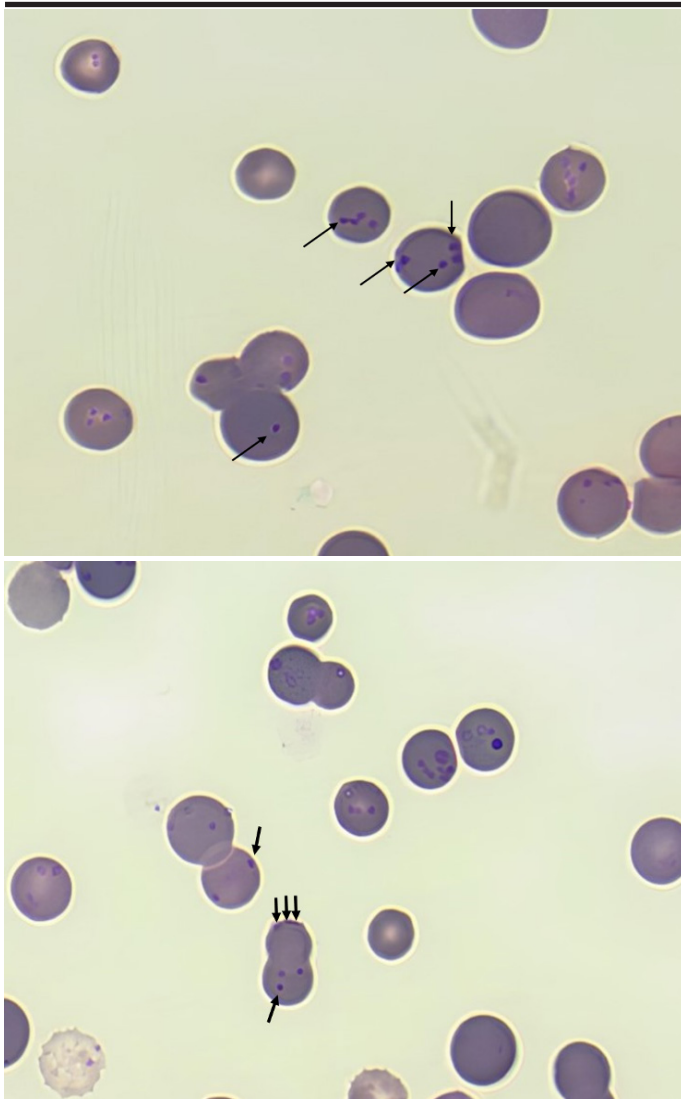


Figure 3. Image of the agent of *Mycoplasma haemofelis* under a light microscope with a 100x objective.

Discussion and Conclusion

Hemotropic mycoplasma infection is the most prevalent etiological factors of hemolytic anaemia in cats. The clinical signs associated with this infection include weight loss, dehydration, tachypnea, tachycardia, pallor of mucous membranes, hyperthermia during the acute phase and hypothermia in cats in the late stages of the disease. It has been documented that the disease more frequently affects male cats under 4-6 years of age, particularly those with free access to outdoor and those not vaccinated against FeLV. The clinical findings in this case, in conjunction with the age, housing and vaccination status of the animal, are consistent with those reported in previous studies (Kurtdele and Ural 2004; Aslan et al. 2010).

The presence of retrovirus infections has been demonstrated to be a risk factor for hemotropic mycoplasmosis in cats, with the potential to result in a more severe presentation of clinical signs. (Bobade et al., 1988, Harrus et al., 2002). The previous

studies showed that hemotropic mycoplasmas induce a significantly more severe anaemia in cats infected with both feline leukaemia virus and feline immunodeficiency virus (Bobade et al., 1988; Messick, 2004). Ural et al. (2008) reported the presence of severe anaemia in a cat naturally infected with "*Candidatus M. haemominutum*", despite the absence of concurrent FeLV or FIV infection. Similarly, despite the absence of either FeLV antigen or FIV antibody, severe anemia was observed in the present case. It has been reported that co-infection with multiple *Mycoplasma* species in cats can lead to increased disease severity, particularly when a cat already infected with one species becomes infected with an additional, different *Mycoplasma* species. (Sykes, 2003). Westfall et al. (2001) demonstrated that cats chronically infected with *Mycoplasma haemominutum* developed significantly more severe clinical signs when subsequently inoculated with *M. haemofelis*, compared to cats with no prior mycoplasma infection that were inoculated with *M. haemofelis* alone. Although immunosuppressive agents such as FeLV and FIV were not detected in the present case, the severe anaemia observed in the cat suggests that the cat may have been infected with more than one *mycoplasma* species.

The most common haematological alterations associated with hemotropic *mycoplasma* infections encompass regenerative anaemia with anisocytosis, reticulocytosis and polychromasia, and the presence of Howell-Jolly bodies (Sykes, 2003; Sykes, 2010). Aslan et al. (2010) found macrocytic and hypochromic anaemia in a cat with hemotropic mycoplasmosis. It has been hypothesised that anaemia resulting from concurrent FeLV and *M. haemofelis* infections may be non-regenerative. However, previous studies have reported macrocytic-hypochromic regenerative anemia, high levels of parasitemia, and poor response to treatment in cats concurrently infected with FeLV and *Mycoplasma haemofelis*. (Bobade et al, 1988; Harrus et al., 2002). In another study, a cat with *Mycoplasma haemofelis* infection without FeLV developed severe hemolytic anemia with a marked increase in reticulocyte count (VanSteenhouse et al., 1995). In the present case, macrocytic-hypochromic anemia observed in hemotropic *Mycoplasma* infection without concurrent FeLV infection is consistent with the previous studies.

Elevated hepatic enzyme activities resulting from hypoxic hepatic injury, along with hyperbilirubinemia caused by hemolysis, are common biochemical alterations observed in cats with hemotropic

mycoplasmosis. Kurtdede and Ural (2004) reported serum concentrations of ALT, AST, total bilirubin, and direct bilirubin as 1053 U/L, 220 +U/L, 1.42 mg/dL and 0.87 mg/dL, respectively, in a cat infected with *M. haemofelis*. In another study, the serum concentrations of ALT, AST, total bilirubin and direct bilirubin were measured as 2703 IU/L, 1822 IU/L, 27.3 mg/dL and 20.3 mg/dL, respectively, in a cat infected with *M. Haemofelis* (Aslan et. al. 2010). The findings of the present study are consistent with those of previous reports. An increase in indirect bilirubin concentration is expected in diseases with hemolytic anemia. However, both in the present case and in the case reported by Aslan et al. (2010) direct bilirubin concentration increased along with indirect bilirubin. This finding may be explained by increased levels of proinflammatory cytokines in hepatocytes due to systemic infection, causing a decrease in the gene expression of transport proteins involved in the passage of direct bilirubin into the bile ducts. Alternatively, the increase may be explained by hepatocyte swelling as a result of hypoxic damage causing pressure on intrahepatic bile ducts (Trauner et al. 1999, Bhogal and Sanyal, 2013). In conclusion, Feline hemotropic mycoplasmosis should be included in the differential diagnosis for cats presented with icterus, increased rectal body temperature and anemia.

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