Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Feline Renal Diseases: Novel versus Traditional Biomarkers

Ghada M. Khalil[1*], Khaled M. A. Mahran[1], Mohamed E. Ali[2] and Shaymaa I. Salem[1]

[1] *Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.*

[2] *Department of Internal Medicine and Infectious Diseases (Internal Medicine), Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.*

Abstract

RENAL diseases are common disorders of felines, considered a leading death cause, and exhibit a diagnostic challenge due to a shortage of early damage markers. The traditional biomarkers [e.g., serum creatinine (sCr) and blood urea nitrogen (BUN)] have low diagnostic sensitivity; thus, acute cases progress to chronic. This study aimed to clarify the clinicopathological, ultrasonographic, and pathological findings of cats affected with renal diseases, and the performance of novel renal biomarkers [symmetric dimethyl arginine (SDMA), kidney injury molecule-1 (KIM-1), Tamm-Horsfall protein (THP), and N-acetyl-β-D-glucosaminidase (NAG)] against the traditional ones. Furthermore, assessing the novel biomarker's role in the follow-up of treated cases. 86 cats were allocated into 4 groups depending on clinical signs, physical examination, sCr concentration, and urine specific gravity (USG) as follows: I. Control negative group (15 apparently healthy cats), II. Control positive group (22 renal diseased cats), III. Pre-azotemic group (31 cats), and IV. Diseased and treated group (18 treated cats). Blood and spot urine samples were collected from all groups. Blood samples for hematological, biochemical, arterial blood gas (ABG) analysis, as well as, SDMA, and KIM-1 concentrations and spot urine samples for routine urinalysis, some biochemical parameters, NAG activity, and THP concentration. Groups II and III showed microcytic hypochromic anemia and significant leukopenia, significant increases in serum concentrations of SDMA, and KIM-1, and activity of urinary NAG; meanwhile, they showed a significant decrease in urinary THP concentration. Group IV after treatment revealed an improvement of all measured parameters except urinary THP concentration. In conclusion, the current study results found out that SDMA, KIM-1, and NAG could serve as efficient biomarkers of early renal injury detection and treatment follow-up.

Keywords: SDMA, KIM-1, NAG, THP, urinalysis.

Introduction

Renal diseases are common in cats [1]. They exhibit a diagnostic and therapeutic challenge due to a shortage of early damage markers. Renal biomarkers could be either traditional markers of kidney function or novel markers of renal injury. The traditional markers; include serum creatinine (sCr), blood urea nitrogen (BUN), and urinalysis. The sCr and BUN have low diagnostic sensitivity for early acute stages of diminished kidney function [2, 3]. Thus, acute cases progress to chronic with significant irreversible damage to the renal parenchyma, which is common in geriatric cats with a prevalence up to 80% and is considered a leading death cause [4].

Routine urinalysis is a highly beneficial and cost-effective diagnostic tool that can indicate an ongoing kidney disease. Assessing several urinary biomarkers is more favorable than relying on a single biomarker and, over a comprehensive urinalysis involves assessing the physical, chemical, and sediment examinations [5]. Time will generate more specific urinary biomarker profiles in various early cases [6].

Novel biomarkers are various biochemical markers that have been proposed as more sensitive indicators of renal injury rather than renal function [7], and thus, they improve the accuracy of diagnosis of renal diseases at earlier stages, allowing early therapeutic intervention and decreasing mortality [2, 3, 8]. They have the potential to reflect the site and severity of renal damage. They can help differentiate between glomerular and tubular involvement, thus improving the diagnosis and staging or grading of renal diseases [6, 9]. These biomarkers could be used for evaluation of glomerular filtration rate (GFR) as symmetric dimethyl arginine (SDMA) or tubular injury as kidney injury molecule-1 (KIM-1), Tamm-

*Corresponding authors: Ghada M. Khalil, E-mail: ghadakhalil@cu.edu.eg, Tel.: 01116314966
(Received 8 September 2024, accepted 13 October 2024)
DOI: 10.21608/EJVS.2024.319153.2366

Horsfall protein (THP), and N-acetyl-β-D-glucosaminidase (NAG) [10]. Whilst KIM-1 and NAG indicate proximal tubular injury, THP is indicative for distal tubular injury and loop of Henle injury.

The GFR biomarker SDMA is a methylated form of arginine found within all nucleated cells. It is released into circulation after proteolysis, then excreted primarily (≥90%) by renal clearance [11]. Therefore, it is a novel renal biomarker considered an endogenous marker of GFR [12]. In 2015, the International Renal Interest Society (IRIS) amended the incorporation of SDMA along with sCr in the guidelines for the diagnosis and treatment of chronic kidney disease (CKD) in cats [13]. The SDMA concentration increases earlier than sCr in cats with acute kidney injury (AKI) and CKD when there is a 25%- 40% decrease in GFR; thus, it is a more sensitive indicator [12]. SDMA is suitable for diagnosing AKI or CKD but could not differentiate between them [14]. It is more specific than sCr as well, being less impacted by extra-renal factors including advanced age and body condition. Therefore, it is more reliable for assessing kidney function in animals with conditions associated with muscle loss, such as feline hyperthyroidism or advanced CKD, in which sCr may underestimate the degree of renal dysfunction [11, 15].

The tubular injury marker, KIM-1, is a proximal renal tubular type I transmembrane glycoprotein [16, 17]. Although KIM-1 is almost undetectable in healthy kidney tissue, it is elevated in all experimental and clinical kidney diseases while proximal tubular epithelial cells become active/injured and overexpress KIM-1 at the apical membrane [18, 19]. Thus, elevated urinary KIM-1 concentration is strongly correlated to injured/necrosed proximal tubules and is a specific excellent novel renal biomarker that predicts tubulointerstitial lesions in AKI [20, 21, 22]. Serum and urinary KIM-1 concentrations were increased in cats with lower urinary tract infections [17]. In addition, KIM-1 is also considered a marker of regenerating tubular cells [23, 24]. A proximal tubular injury biomarker, NAG, is a hydrolytic lysosomal enzyme with a high molecular weight and very low physiological activity [25]. NAG is plentifully expressed in the proximal renal tubular epithelium. Under normal circumstances, it is secreted in small amounts, maintaining a low urinary NAG activity. However, when the renal tubular epithelium is damaged, its secretion is enhanced, causing significantly increased urinary activity [10, 26]. In different renal diseases, urinary NAG activity was found to be increased before the sCr concentration or urinary protein/creatinine ratio (UPC) increment [22, 27]. Thus, urinary NAG activity is used for early detection of renal damage, particularly AKI in cats [8].

The distal tubular injury marker, THP (MW 100 kDa), is synthesized by the cells of the thick ascending loop of Henle and the distal convoluted tubule and is located predominantly on the surface of the luminal cell membrane [10]. THP represents nearly 50% of total proteins that are usually lost in the urine of healthy cats, and its biological function is still not fully understood [6, 28], however, it is believed to have roles in water and electrolyte balance in Henle's loop, protection against urinary tract infection, and some immunomodulatory activity; nonetheless, it is clinically important as it represents the matrix of all urinary casts. Normal urine has high concentrations of THP, while significantly reduced urinary THP concentrations were seen in cats with renal disease [9]. Therefore, the evaluation of THP as an early AKI biomarker is potentially valuable [6].

This study aimed to clarify the clinicopathological, ultrasonographic, and pathological findings of cats affected with renal diseases and to evaluate the performance of novel biomarkers of kidney injury (SDMA, KIM-1, NAG, and THP) against traditional biomarkers of kidney function for diagnosing renal diseases in cats during their initial phase. Furthermore, assessing the novel biomarker's role in the follow-up of treated cases. In addition, measuring the correlation between those novel renal biomarkers versus sCr and some urinary measurements [protein, microalbumin, and urine specific gravity (USG)].

Material and Methods

Animals

The current study was carried out between 2020 to 2023 on 86 cats with an average age 7± 2.5 years and weight 3.75± 1.25 kg. These cats were admitted to the clinic of the Internal and Infectious Diseases Department, Faculty of Veterinary Medicine, Cairo University, and some clinics and shelters in Giza and Cairo governorates. Cats were allocated into 4 groups depending on clinical signs, physical examination, sCr concentration, and USG according to [8, 29] as follows: I. Control negative group (15 apparently healthy cats): those had sCr=1.21± 0.27 mg/dL and USG=1.036± 0.02. II. Control positive group (22 renal diseased cats): those suffered a diversity of clinical signs, included vomiting, diarrhea, polydipsia, polyuria, different oliguric phases in some cases, and pale mucous membrane, as well, they had sCr=5.25± 1.29 mg/dL and USG=1.020± 0.06. III. Pre-azotemic group (31 cats): those had sCr=1.59± 0.14 mg/dL, USG =1.035± 0.01 [29, 30]. IV. Diseased and treated group (18 treated cats): those were treated using patent drugs: Azodyl® (Vetoquinol, France) with a dose of 2 capsules daily (1 capsule a.m., 1 capsule p.m.) and Ipakitine® (Vetoquinol, France) with the dose of 1 g/5 kg body

weight, once in the morning and once in the evening with the meal, for 2 weeks and followed up [31]. Cats included in the current study were exposed to complete physical and ultrasonography examinations, then blood and urine samples were collected for laboratory examinations.

Ultrasonography

Ultrasonographic examination of the kidneys and urinary bladders from different groups was performed using a C11-3s microconvex transducer (Mindray M9, China) according to the procedure described by [32].

Sampling

Blood and urine samples were collected from all cats under study. Moreover, kidney tissue samples were collected from a cat that died in a veterinary clinic and used for postmortem pathological examination.

A venous blood sample of 3 ml volume was collected from the jugular vein and allocated into three parts: one on ethylene diamine tetra acetic acid (EDTA) as whole blood for hematological examination, another part on lithium heparin for arterial blood gas analysis (ABG), and the last part on a plain tube for serum separation for biochemical exanimation.

Urine samples were collected via manual compression of the urinary bladder, examined physically and chemically by dipstick, and then centrifuged at 400×g [5]. The supernatant was used for biochemical and enzyme-linked immunosorbent assay (ELISA) analysis, while the sediment was examined microscopically for cytological evaluation.

Clinicopathological examination

Hematological examination

Complete blood count (CBC) included red blood cells (RBCs) count, hemoglobin (Hb) concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and total leukocytic count (TLC) were evaluated by Animal Cell Counter (ABC Vet, France). Differential leukocytic count (DLC) was examined according to [33].

Arterial Blood Gas (ABG) analysis

Heparinized blood samples were immediately transported in an ice box to the laboratory and analyzed by a blood gas analyzer (GEM Premier 3000, USA) for acid-base balance indices, which included blood pH, partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), chloride (Cl^-) concentration, and bicarbonate (HCO_3^-) concentration.

Biochemical examination

Traditional parameters

Serum samples were used to evaluate traditional kidney function biomarkers, which include sCr and BUN concentrations, protein profiles, and concentrations of serum electrolytes and minerals.

The protein profile includes total proteins, albumin, and globulins concentrations as well as albumin globulins (A/G) ratio. The serum electrolytes and minerals include calcium, phosphorus, sodium, and potassium concentrations. All the mentioned parameters were evaluated according to the manufacturer (Spectrum, Egypt).

Spot urine samples were used for routine urinalysis, spot urinary creatinine concentration (uCr), protein concentration, urinary protein/creatinine ratio (UPC), microalbumin concentration, sodium concentration, fractional excretion of sodium (FENa), and activity of urinary gamma-glutamyl transferase (uGGT). Routine urinalysis included physical examination, measurement of specific gravity by handheld refractometer (HRM18-T, KRÜSS, Germany), dipstick analysis (Medi-Test Combi 10 SGL, MACHEREY-NAGEL, Germany), and sediment cytology by manual microscopy according to [5]. For the UPC ratio, urinary creatinine concentration was measured with the Jaff method after dilution of the urine sample 50 times, protein concentration was measured with the pyrogallol-red method, then the UPC ratio was calculated according to [34], microalbumin concentration was measured by the turbidimetry method. All mentioned parameters were evaluated according to the manufacturer (Spectrum, Egypt). FENa was calculated according to [35] as follows:

$$\text{FENa}(\%) = \frac{(Urine\ Na\ concentration)x(Plasma\ creatinine\ concentration)}{(Urine\ creatinineconcentration)x(Plasma\ Na\ concentration)}\ x\ 100$$

Novel parameters

Novel renal biomarkers were determined using specific feline ELISA kits (BT LAB, China). These biomarkers included serum SDMA (catalogue No. E0022Cat), serum KIM-1 (catalogue No. E0097Cat), urinary NAG (catalogue No. E0023Cat), and urinary THP (catalogue No. E0113Cat).

Pathological examination

The kidneys from a dead cat of group II were grossly examined, then the tissue was fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned, and stained with Hematoxylin and Eosin (H& E) according to [36].

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for

Windows version 25.0 (SPSS Inc., USA); significance was set at $p \leq 0.05$. Quantitative variables were presented as Mean ± SD and statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison post hoc test for different groups. Group IV quantitative variables were statistically analyzed by dependent sample t test. The Pearson's correlation coefficient was used to measure the correlation between novel renal biomarkers: SDMA, KIM-1, NAG, and THP versus sCr and some urinary measurements (proteinuria, MAU, and USG). Correlation strength was defined as follows: weak: r = 0.0–0.39; moderate: r = 0.4–0.69; strong: r = 0.7–1.0 [37].

Results

Ultrasonographic Findings

Ultrasonographic examination of cats' kidneys and urinary bladders of groups I and III showed normal echogenicity, size, and thickness of the tissues without any masses, cysts, or lesions. Group II showed different abnormal findings, including a slightly enlarged kidney with mild loss of corticomedullary distension and multiple cysts (Fig. 1, c). Some cases showed small irregular kidneys with loss of corticomedullary distinction. Dilated renal pelvis compressing the normal kidney tissue architecture was observed in some cases. As well, other cases showed enlarged hyperechoic cortex with loss of corticomedullary distinction of the kidney. Urinary bladders in some cases exhibited moderate thickening of the wall with moderate echogenic multiple sediments inside the lumen, as shown in (Fig. 1, d).

Clinicopathological Findings of Different Groups (I, II and III)

Clinicopathological findings of different groups are illustrated in (Table 1.) Erythrogram results revealed a significant decrease in the RBCs count, Hb concentration, Hct, MCV, and MCHC, representing a microcytic hypochromic anemia of group II (control positive group) compared to group I (control negative group). Meanwhile, in comparison to group I, group III (pre-azotemic group) showed a significant decrease in Hb concentration, Hct, MCV, and MCHC, representing a microcytic hypochromic anemia, and non-significant change in RBCs count.

Leukogram results revealed a significant decrease in TLC due to a significant decrease of neutrophils, monocytes, and eosinophils associated with a significant increase of lymphocytes in groups II and III compared to group I.

The ABG revealed a significant pH decrease in group II, indicating acidosis, and a non-significant difference in group III when compared to group I. PCO2 and PO2 showed non-significant change among the different groups. Chloride concentration showed a significant increase in group II and a non-significant change in group III; meanwhile, HCO3- concentration showed a significant decrease in group II and a non-significant change in group III compared to group I.

Traditional renal biomarkers showed a significant increase of BUN concentration in group II compared to group I, along with sCr concentration and USG, confirming azotemia in group II. BUN concentration showed a significant decrease in group III compared to group II, meanwhile it showed a non-significant change when compared to group I; thus, group III cats were non-azotemic.

The protein profile exhibited a significant decrease in serum total proteins concentration due to a significant decrease in globulins concentrations with a non-significant change in albumin concentration, as well as a significant increase in the A/G ratio of group II when compared to group I. Furthermore, a non-significant difference was found in the protein profile in group III when compared to group I.

Electrolytes and minerals revealed a non-significant change of calcium concentration among different groups, a significant increase of phosphorous concentrations in group II, and a non-significant change in group III when compared to group I. A significant decrease of sodium and a significant increase of potassium concentrations occurred in group II compared to group I; meanwhile, non-significant change occurred for both in group III compared to group I.

Novel serum renal biomarkers showed significant increases of SDMA and KIM-1 concentrations in groups II and III compared to group I

Clinicopathological Findings of Group IV (before and after treatment)

Clinicopathological findings of group IV were illustrated in (Table 2.) Before treatment, they were similar to that of group II when compared to group I. However, erythrogram after treatment revealed a significant increase of the RBCs count, Hb concentration, and Hct with a non-significant change of MCV and MCHC when compared to their values before treatment.

Leukogram after treatment revealed a significant increase of TLC due to a significant increase of neutrophils, monocytes, and eosinophils associated with a significant decrease of lymphocytes when compared to their count before treatment.

ABG after treatment revealed non-significant changes in all ABG parameters except HCO3- concentration, which showed a significant increase compared to their concentrations before treatment.

Traditional renal biomarkers after treatment exhibited a significant decrease in sCr and BUN concentrations, thus confirming an improvement in renal function.

The protein profile after treatment was improved and showed a significant increase in total proteins and globulins concentrations, a significant decrease in the A/G ratio, and a non-significant change in albumin concentration compared to their concentrations before treatment.

Electrolytes and minerals concentrations after treatment showed a significant decrease of calcium and potassium concentrations, and a non-significant change in concentrations of phosphorous and sodium compared to their concentrations before treatment.

Serum SDMA and KIM-1 concentrations showed a significant decrease compared to their concentrations before treatment. Thus, improvement of the renal function, indicated by reduced concentrations of sCr and BUN, is accompanied by improvement of SDMA of KIM-1 concentrations.

Complete Urinalysis Findings of Different Groups (I, II and III)

Complete urinalysis findings for different groups are shown in (**Table 3.**) Physical examination of the urine samples in all cases of group I and the majority of cases in-group III revealed yellow colour, urinepherous odour, and a clear aspect. In contrast, in group II, urine samples appeared red with an ammoniacal odour, and a turbid aspect. Urine specific gravity (USG) exhibited a significant decrease in group II and a non-significant change in group III compared to group I; however, urine pH showed a non-significant change among the different groups.

Chemical examination of the urine samples in all cases of group I and the majority of cases in group III showed absences of protein, blood, and leukocytes, while in group II, urine samples exhibited proteinuria, hematuria, and pyuria.

Microscopical examination of the urine sediment of all group I cases and the majority of group III cases showed normal RBCs and pus cells, with the absence of crystals and casts, while in group II, samples revealed an increased number of RBCs and pus cells with the presence of crystals “crystalluria” (triple phosphate and calcium oxalate crystals).

The urinary biochemical parameters revealed a significant increase of urinary proteins, UPC, microalbumin, sodium (Na), FENa, and uGGT, and a significant decrease of spot uCr concentration in group II compared to group I. Furthermore, in comparison to group I, group III showed a significant increase of urinary proteins, UPC, microalbumin, and uGGT and a non-significant change of sodium and FENa with a significant decrease of uCr.

Novel urinary renal biomarkers showed a significant increase of urinary NAG activity and a significant decrease of THP concentration in groups II and III compared to group I.

Correlation between Serum Novel versus Traditional Standard Biomarkers

Pearson's correlation coefficient analysis of novel serum biomarkers showed that there was a strong positive correlation between sCr and SDMA concentrations ($r=0.764$, $p<0.0001$), a moderate positive correlation between sCr and KIM-1 concentrations ($r=0.604$, $p<0.0001$), and a strong positive correlation between SDMA and KIM-1 ($r=0.702$, $p<0.0001$). In addition, proteinuria had a strong positive correlation with sCr concentration ($r=0.816$, $p<0.0001$) and SDMA concentration ($r=0.840$, $p<0.0001$) and a moderate positive correlation with KIM-1 concentration ($r=0.556$, $p\leq0.01$). Also, MAU had a very strong positive correlation with sCr concentration ($r=0.925$, $p<0.0001$), a strong positive correlation with SDMA concentration ($r=0.793$, $p<0.0001$), and a moderate positive correlation with KIM-1 concentration ($r = 0.615$, $p<0.001$). Urine specific gravity had a moderate negative correlation with sCr, SDMA, and KIM-1 [($r=-0.556$, $p\leq0.01$), ($r=-0.586$, $p\leq0.01$), and ($r=-0.46$, $p\leq0.05$), respectively].

Complete Urinalysis Findings of Group IV (before and after treatment)

Complete urinalysis findings for group IV are shown in (Table 4.) Physical examination of the urine samples in the majority of cases in group IV before treatment appeared red with an ammoniacal odour, and turbid aspect, while after treatment all cases revealed yellow coloured urine with a urinepherous odour, and clear aspect. Urine specific gravity revealed a significant increase after treatment compared to its concentration before treatment; however, urine pH showed a non-significant change.

Chemical examination of the urine samples in the majority of cases in group IV before treatment exhibited proteinuria, hematuria, and pyuria, while after treatment the majority of cases showed absences of protein, blood, and leukocytes.

Microscopical examination of the urine sediment in the majority of cases of group IV before treatment revealed an increased number of RBCs and pus cells with crystalluria (triple phosphate and calcium oxalate crystals), while after treatment all cases showed normal number of both RBCs and pus cells with the absence of crystals.

The urinary biochemical parameters of group IV before treatment were similar to those of group II when compared to group I; however, after treatment they revealed a significant increase of spot uCr, a significant decrease of protein, UPC, microalbumin, and FENa, and a non-significant change in sodium

and uGGT when compared to their values before treatment.

Novel urinary renal biomarkers concentrations were similar to that of group II when compared to group I; however, after treatment they showed a significant decrease of NAG activity, and a non-significant change of THP concentration compared to their result before treatment.

Correlation between Urinary Novel versus Traditional Standard Biomarkers

Pearson's correlation coefficient analysis of novel urinary renal biomarkers showed that there was a moderate positive correlation between NAG activity and sCr concentration ($r=0.634$, $p\leq0.01$), a moderate negative non-significant correlation between the THP concentration and sCr concentration ($r=-0.415$, $p\leq0.07$), and a moderate negative correlation between NAG activity and THP concentration ($r=-0.554$, $p\leq0.01$). In addition, proteinuria had a strong positive correlation with NAG activity ($r=0.756$, $p<0.0001$) and a moderate negative correlation with THP concentration ($r=-0.507$, $p\leq0.05$). Also, MAU had a strong positive correlation with NAG activity ($r=0.702$, $p<0.0001$) and a moderate negative correlation with THP concentration ($r=-0.481$, $p\leq0.05$). Urine specific gravity had a moderate negative correlation with NAG activity ($r=-0.526$, $p\leq0.05$) and a moderate positive correlation with THP concentration ($r=0.514$, $p\leq0.05$).

Pathological Findings

Gross Findings

Postmortem examination of a dead cat from group II revealed a marked decrease in the size of both kidneys with adhered capsules. Both kidneys were pale in colour and firm in texture.

Histopathological Findings

Photomicrographs of H& E-stained renal tissue of a dead cat from group II (Fig. 2) revealed extensive scarring that extends into cortical and medullary zones of the kidney. Interstitial fibrosis with distortion of tubules, as some of the renal tubules were atrophied and had reduced luminal diameter, and others appeared ectatic, lined by flattened epithelium, producing cystic dilatation with homogeneous proteinaceous casts. Also, renal tubules suffered from tubulitis with necrobiotic changes in their lining epithelium. Interstitial tissue was infiltrated with lymphoplasmacytic cells. The renal corpuscles have contracted glomeruli with thickened hyalinized Bowman's capsules and periglomerular fibrosis. Renal tophi of urate crystals were deposited in the interstitium and intratubular lumen. Arteriolonephrosclerosis was detected with vacuolation of media and fragmentation of elastic lamina.

Discussion

This study aimed to evaluate the performance of novel biomarkers (SDMA, KIM-1, NAG, and THP) in cats versus traditional biomarkers for detecting kidney injury and assess their role in the follow-up of treated cases.

Ultrasonographic findings of group I and III kidneys showed normal echogenicity, size, and thickness of cortex and medulla with distinct corticomedullary junction. Meanwhile, group II showed different abnormal findings including slightly enlarged kidneys, which contributed to the presence of multiple cysts with mild loss of corticomedullary distension, a case known as polycystic kidney disease (PKD). PKD's cysts are several thin-walled ovoid structures of varying diameters characterized by anechoic content [38]. The causes of PKD are mostly congenital [39]. Other cases showed small irregular kidneys with loss of corticomedullary distinction due to decreased cortical thickness in cases of CKD [40]. Meanwhile, an enlarged hyperechoic cortex with loss of corticomedullary distinction is a characteristic of acute nephritis, which may be attributed to interstitial nephritis and gradual loss of nephron function [39]. Renal pelvic dilation as a result of obstruction is typically the indication for hydronephrosis, and substantial tissue loss may occur as a consequence of pressure necrosis in the renal parenchyma. A thin border surrounding a significantly dilated pelvis may be the only remaining tissue in extreme cases [41]. Ultrasonographic findings of groups I and III urinary bladders revealed a normal wall thickness, and a lumen filled with echoic urine. Urinary bladders of some cases in group II exhibited moderate bladder wall thickening, which may be attributed to a degree of cystitis, and moderate echogenic multiple urine sediment inside the lumen is characteristic for cystic urolithiasis [38, 41].

The sCr and BUN are metabolic waste products that are excreted from the kidneys; the blood concentration of both increases in different renal diseases [29]. Beside the clinical signs and physical examination findings, sCr, BUN and USG were evaluated in the current study to allocate cats in different groups. Significant increase of sCr and BUN with significant decrease of USG confirm the azotemic state accomplished group II (the renal diseased cases), while group III (the pre-azotemic group) showed non-significant change of sCr, BUN and USG confirming their non-azotemic state. Thus, the novel renal biomarkers' competence to detect early stage of renal injury can be assessed in group III. Decreased sCr and BUN after treatment of group IV may suggest hypertrophy alterations in the glomerular apparatus of the kidneys, leading to an enhancement in their excretory function [42].

Hematological evaluations performed in the current study revealed the presence of microcytic hypochromic anemia in groups II and III. This type of anemia is associated with the inflammatory states (in both acute and chronic renal diseases), which contribute to a relative iron deficiency by inducing hepcidin production. Hepcidin is an acute-phase protein that hinders the release of iron stored in macrophages and hepatocytes, thus eventually leading to anemia by reducing the bioavailability of iron for hemoglobin production. In addition, inflammatory cytokines and uremic toxins can adversely affect RBCs survival, causing hemolysis [43, 44]. Gastrointestinal mucosal bleeding and hematuria may contribute to the anemia [43, 45], these observations agreed with [46].

The significant leukopenia in groups II and III may be due to compromised bone marrow function as they had low count of neutrophils, eosinophils, monocytes, and lymphocytes; lymphocytosis was an exception of group II, similar to findings of [47], who conducted a study on collected data of elderly over 3 years from the CKD Research of Outcomes in Treatment and Epidemiology (CKD-ROUTE) and observed a correlation between leukopenia and CKD progression; in addition, Krofič Žel et al., 2024 [48] found that more neutropenic cats were found in early CKD group than in advanced CKD group. Furthermore, oxidative stress and the increased apoptosis of neutrophils can lead to neutropenia and a decline in their functionality; moreover, lymphopenia was accounted for as an accelerated lymphocyte apoptosis [49] and uremia [39]. Lymphocytosis in group II may reflect reactive or transient lymphocytosis; this finding agreed with [50] and disagreed with the finding of [49], who mentioned that lymphopenia was observed more frequently in cats suffering from end-stage renal disease. Monocytopenia can happen whenever there is neutropenia due to the common bipotential stem cell for both [48].

In the current study, group II exhibited metabolic acidosis, which resulted from increased concentrations of uremic acids [51], the inability of the kidney to eliminate nitrogen molecules [52], and H+ with reduced production and reabsorption of HCO3- [53]. Bicarbonate-assisted metabolic acidosis correction permits an intracellular shift of K+ in exchange with H+, which is combined with HCO3- and eliminated [53], explaining the significant decrease of HCO3-. Hyperchloremia of group II may be due to metabolic acidosis from mineral acids (e.g., NH4CL or HCL) [53]. Group IV after treatment showed a non-significant change in ABG parameters compared to their concentrations before treatment except for the significantly increased HCO3-, which may be due to the enhancement of renal tubular reabsorptive function [53] and is considered a compensatory mechanism for the metabolic acidosis state of the diseased cases.

Reported hyperphosphatemia in group II resulted from decreased renal excretion of phosphorous associated with reduced GFR since phosphate is freely filtered at the glomerulus; this finding was similar to that of [4, 52]. Hyperkalemia detected was owing to a reduction in GFR which has a direct impact on the capacity of tubules to retain and reabsorb water and salts, as well as the excretion of potassium, resulting in the development of uremia, acidosis, and hyperkalemia that was similar to findings of [52]. Extracellular pH directly influences cell membrane excitability and stability, boosting potassium efflux and aggravating pre-existing hyperkalemia [52]. In addition, hyperkalemia usually accompanied CKD with oliguria and metabolic acidosis, as exhibited in the current study, which triggers translocation of potassium out of cells in exchange with hydrogen ions entering the cells [53]. Hyponatremia of group II indicates excess sodium loss and may be associated with vomiting [53]. Metabolic acidosis correction with HCO3- allows an influx of K+ intracellularly as the H+ is combined with HCO3- and removed [53], which explains the significant hypokalemia in group IV after treatment.

Hypoproteinemia in group II is associated with anemia, suggesting anemia of chronic disease [50]. Hypoproteinemia develops in many cats with glomerular disease due to hypoalbuminemia [54], which results from inflammation as a negative acute phase protein and/or protein-losing nephropathies associated with heavy proteinuria [55]. However, in the current study, hypoalbuminemia in group II may be masked by dehydration. Hypoglobulinemia with normal albumin occurs due to acquired immune deficiency [56]. This finding disagreed with [50], who revealed mild hyperglobulinemia suggesting mild subclinical inflammation.

Serum SDMA originates from the intranuclear methylation process of L-arginine facilitated by protein arginine methyltransferase. It is predominantly excreted through the kidney, indicating its potential as an endogenous marker for glomerular filtration rate (GFR) [8]. The production rate of SDMA in the body is relatively stable, but protein breakdown could potentially contribute to changes in serum concentration [30]. Serum SDMA concentrations were shown to be significantly increased in group II due to glomerular pathological affections that cause a decrease in GFR and an elevation in the serum concentrations of SDMA [57]. This finding was identical to the findings of [57, 58], who found that serum SDMA concentrations were increased above the normal reference range before elevation of sCr concentrations above their normal reference range. SDMA concentrations for group III were found to be increased in association with non-significant changes of both sCr and BUN

concentrations, that proving the sensitivity of SDMA is significantly higher than sCr and BUN. The current study finding disagreed with Wun et al., 2024 [59], who concluded that under their experimental conditions, there is no evidence to suggest that serum SDMA is more effective than sCr in detecting impaired renal function caused by meloxicam-induced renal injury in cats. However, SDMA concentration in group IV after treatment was significantly decreased than before treatment; this may be facilitated by an increase in glomerular filtration [42], revealing its usefulness as a follow-up marker for renal repair.

The KIM-1 is a 38.7 kDa type I transmembranous glycoprotein with immunoglobulin and mucin structural domains. Elevated serum KIM-1 was found shortly after renal tubular injury [10]. The current study revealed a significant increase of KIM-1 in group II, confirming renal tubular injury, similar to findings of Xavier Júnior et al., 2022 [60], who quantified KIM-1 in feline urine to evaluate it as a biomarker of AKI, and identical to findings of Elkhiat et al., 2019 [17], who measured serum KIM-1 concentration in cats affected with lower urinary tract disease and concluded that its increase may be due to renal tubular impairment after acute renal failure. KIM-1 concentrations of group III were increased in association with non-significant change of both sCr and BUN concentrations, proving that the sensitivity of KIM-1 is significantly higher than both sCr and BUN. Concentration of KIM-1 was significantly decreased in group IV after treatment compared to its concentration before treatment, establishing its convenience as a follow-up marker for renal repair. Zou, Wang, and Lu, 2022 [61] illustrated that correlating uKIM-1 and sKIM-1 can improve the accuracy and precision of detecting severe acute tubular necrosis, and this enables early treatment. Thus, further studies measuring and correlating serum and urinary KIM-1 in cats are required.

Physical examination of the urine samples of group II revealed that urine samples appeared red with ammoniacal odour, and turbid aspect. Red urine may be caused by hematuria (presence of intact erythrocytes in the urine) or hemoglobinuria due to lysed erythrocytes either prerenal or in dilute or alkaline urine; both react positive for blood on a urine dipstick [62]. Hematuria, confirmed by microscopic sediment examination and revealed that RBCs were > 5/HPF, may be attributed to inflammatory renal disease or disease of the lower urinary tract [5]. Our findings were similar to that reported by [39]. Despite the fact that urine odour may not be considered pathognomonic for any disease, it is found that ammoniacal odour suggests urinary infections mostly with urease-positive bacteria [62]. Under disease conditions, urine turbidity is associated with an increase in the number of cells, crystals, deposits, and/or organisms [62]. Physical examination of the urine samples in the majority of cases in group IV after treatment showed normal yellow coloured urine with urinepherous odour, and a clear aspect indicating relief of the urinary tract infection and inflammation [62].

The USG is a metric that quantifies the weight ratio of 1 L urine to 1 L water. It serves as a crucial indicator of the kidney's capacity to concentrate or dilute urine [62]. USG decrement of group II indicates reduced urine concentrating ability by kidneys and holds clinical significance serving as an early indicator of kidney disease; this finding was identical to findings of [63, 64]. Group IV after treatment showed a significant increase of USG, confirming that their kidneys retained the concentrating ability of urine [62].

The chemical examination of most urine samples in group II exhibited proteinuria, hematuria, and pyuria. Hematuria associated with substantial pyuria can give rise to concomitant mild-to-moderate proteinuria by dipstick [5]. Microscopical examination of most urine samples' sediment of group II revealed active sediments, an increased number of RBCs and pus cells in association with crystalluria (triple phosphate and calcium oxalate crystals). Active sediment with proteinuria indicates of inflammatory kidney or urogenital disease [5]. Crystals in urine, or crystalluria, are common even in healthy states. However, struvite crystal is mostly found in basic urine, and calcium oxalate monohydrate crystal increases suspicion of ethylene glycol poisoning [62]. Most cases in group IV after treatment showed absences of protein, blood, and leukocytes. All cases in group IV after treatment showed normal number of both RBCs and pus cells without presence of crystals owing to increased renal integrity.

Significant proteinuria in groups II and III may be due to a damaged barrier of the glomerular filtration system, the inability of the tubules to completely reabsorb filtered proteins, and/or leakage of proteins and enzymes from tubular epithelial cells [8, 9]. This agreed with the findings of Ribeiro et al., 2020 [63], who performed a transversal study in dogs suffering from CKD and observed that the higher the protein loss, the higher the stage of the disease was. High urine protein and low specific gravity were linked to more severe acute illness and the onset of AKI, according to a human study on urinalysis [65]. Proteinuria in most cases of group IV after treatment was significantly decreased than its concentration before treatment, thus indicating an enhancement in the kidney's concentration function. This improvement may be linked to the hypertrophy of intact nephrons [42].

Spot uCr concentrations in groups II and III were significantly decreased, which accounts for impaired

renal excretory function for creatinine, identical to the findings of [60]. Spot uCr concentration in group IV after treatment was significantly increased, accounting for enhanced renal excretory function for creatinine.

The UPC is a commonly employed technique for quantifying proteinuria in the veterinary routine as a substitute for the 24-hour urinary protein excretion measurement and has been validated in cats [66]. Creatinine production remains constant daily; however, its urinary concentration is consistently variable with urine volume. This condition can be mitigated by dividing the urinary protein level (in mg/dl) by the urinary creatinine level (in mg/dl) [63, 67]. The significant increase of UPC in groups II and III disagreed with Williams, and Archer, 2019 [68], who clarified that UPC, and urinary albumin excretion are not increased in cats with early azotemic CKD. Thus, further research is required to examine the biological variability of UPC in cats with kidney diseases and overt renal proteinuria, as well as in cats with elevated UPC ratios [64]. The presence of varying degrees of microscopic hematuria may have played a role in the observed difference in UPC. UPC in group IV after treatment was significantly decreased than that before treatment indicating a deceleration of disease progression and the progress of glomerular fibrosis [69].

The MAU, in which small amounts of albumin are present in the urine, is defined as a urinary albumin concentration >1 and ≤30 mg/dL when urine samples are diluted to a standard specific gravity of 1.010 [6], and thus is considered the earliest sign and the best predictor of renal failure progression [70]. Similarly, in the current study, microalbumin concentration was elevated in groups II and III. Microalbumin concentration of group IV after treatment was significantly decreased than its concentration before treatment; that was accounted for by the hypertrophy of intact nephrons, especially intact glomerular tuft [42]. As well, this makes microalbumin a good marker to follow-up renal function improvement.

Urinary sodium was significantly increased in group II, which is supposed to result from increased sodium excretion and decreased sodium reabsorption per nephron to compensate for the decrease in the number of functioning nephrons and decreased GFR, especially when sodium intake remains constant [71]. Fractional excretion of an electrolyte (FE) is the process of determining the percentage of urinary electrolyte excretion relative to its serum concentration, with the correction made for creatinine excretion based on filtration rate [72]. Under the influence of aldosterone, healthy kidneys reabsorb excess sodium to restore water balance [35]. As the kidney tubules are involved in the reabsorption and secretion of electrolytes, a significant increase in FENa indicates that the cases suffered from tubular impairment [72]. Despite the non-significant change of urinary sodium concentration of group IV after treatment compared to its concentration before treatment, FENa was significantly decreased after treatment owing to the significant decrease of sCr concentration and significant increase of spot uCr after treatment and their effect on FENa equation [35].

The uGGT activity was significantly elevated, which may be due to acute tubular damage as it is located in the metabolically active region of the proximal renal tubules. This was identical to the findings of [65] and Xavier Júnior, et al., 2022 [60], who studied uGGT activity in urethral-obstructed cats and found that its activity was elevated immediately after the diagnosis of the cases. The uGGT activity of group IV after treatment showed a non-significant change compared to its activity before treatment, thus it couldn't be used as a follow-up biomarker for improvement of renal function.

The NAG is a hydrolytic lysosomal enzyme plentifully expressed in the renal proximal tubular epithelium and normally cannot pass through the glomerular filtration. Under normal circumstances, it is secreted in small amounts, maintaining a low urinary NAG activity [22]. The current study revealed a significant increase of NAG activity in group II, as when kidney tubular cells are damaged or injured, they leak NAG into the urine; that was identical to the finding of [73]. In addition, NAG activity for group III was found to be elevated, establishing that the sensitivity of NAG is significantly higher than sCr and BUN. NAG activity significantly declined in group IV after treatment compared to its activity before treatment, indicating decreased leakage by injured tubular cells [22], establishing its usefulness as a follow-up marker for renal repair.

The THP (MW 100 kDa) is synthesized by the cells of the thick ascending loop of Henle and the distal convoluted tubules [9]. Recent studies have revealed that THP concentrations are lower in patients with acute kidney injury than healthy individuals [10]. The current study revealed a significant decrease of THP in group II, indicating damage of the thick ascending loop of Henle and the distal convoluted tubules, identical to the findings of Crisi et al., 2020 [74], who studied the early renal damage in cats infected with Feline Morbillivirus (FeMV), and Ferlizza et al., 2020 [75], who conducted research on dogs with stage I non-proteinuric CKD showed declined THP concentration and figured out that it was a potential early indicator of renal failure in dogs. Precisely, group III showed a significant decrease of THP concentration, confirming that it is a potential marker for early renal tubular damage in cats. Group IV after treatment exhibited a non-significant change in

concentration of THP when compared to its concentration before treatment; thus, it is not useful as a follow-up marker for renal function enhancement.

The strong positive correlation between SDMA and KIM-1 concentrations, in addition to the strong positive correlation of SDMA with sCr and the moderate positive correlation of KIM-1 with sCr concentrations exhibited by Pearson's correlation coefficient analysis, confirm that they are reliable indicators as early serum biomarkers for renal injury. This result is supported by the strong positive correlation of proteinuria as well as MAU with sCr and SDMA and their moderate positive correlation with KIM-1 concentrations. In addition to the moderate negative correlation of USG with sCr, SDMA, and KIM-1.

The moderate negative correlation between NAG activity and THP concentration, in addition to the moderate positive correlation of sCr with NAG and the moderate negative non-significant correlation of sCr with THP exhibited by Pearson's correlation coefficient analysis, confirm that they are reliable indicators as early urinary biomarkers for renal injury. This result is supported by the strong positive correlation of proteinuria and MAU with NAG activity and THP concentration. In addition to the moderate negative correlation of USG with NAG activity and the moderate positive correlation with THP concentration.

Interstitial fibrosis with distortion of tubules found in a renal histopathologic section of a dead cat of group II is caused by capillary rarefaction, the reduction in the number of capillaries, which is strongly associated with fibrosis as it is regarded as a significant factor in the development of hypoxia and the progression of fibrosis [76]. That was similar to the findings of Paschall et al., 2023 [77], who assessed the renal peritubular capillary rarefaction of CKD. Fibrosis is most likely triggered by injury to tubular epithelial cells and pericytes, leading to either death or transdifferentiation into myofibroblasts that produce extracellular matrix [78]. McLeland et al., 2015 [79] added that cats with CKD exhibit inflammation and scarring in the tubules and interstitial tissue, as well as atrophy of the tubules and the development of glomerulosclerosis as a subsequent effect.

The interstitial fibrosis that occurs as a result of this process increases the distance that oxygen needs to diffuse between capillaries and tubular epithelial cells, leading to ischemia injury and further damage [78, 80]. Ultimately, this results in tubular atrophy and the loss of nephrons. The loss of nephrons intensifies the workload on the remaining ones and worsens the condition of renal hypoxia, which affects tubular epithelial cells in particular [78]. Arteriolonephrosclerosis is associated with loss of functioning glomeruli and causes hyperfiltration of remaining glomeruli, leading to podocyte damage and sclerosis that was identical to findings of [79].

Conclusion

The current study results found out that SDMA concentration, KIM-1 concentration, and NAG activity were significantly increased, while THP concentration was significantly decreased in the pre-azotemic group of cats having sCr and BUN concentrations near the upper reference ranges. Thus, they could serve as efficient early renal biomarker indicators of renal injury. In addition, SDMA concentration, KIM-1 concentration, and NAG activity were significantly declined in the treated group of cats, ensuring their usefulness as follow-up biomarkers for follow-up marker for renal repair and improvement of kidney function. On contrast, THP concentration wasn't changed significantly after treatment; therefore, it couldn't be used as a follow-up biomarker for renal repair or improvement of kidney function. Combining glomerular markers, such as SDMA and proteinuria, with tubular markers, such as KIM-1, NAG, and USG, could yield a valuable 'biomarker panel' to enable earlier detection of renal disease, enhance the follow-up of cases, and permit more precise surveillance of therapy response.

Acknowledgments

Not applicable.

Funding statement

The study supported by Cairo University research funds for postgraduate studies.

Declaration of Conflict of Interest

There are no conflicts to declare

Ethical of approval

This study was performed by local animal ethics guidelines. It was approved by the Institutional Animal Care and Use Committee of Cairo University (IACUC) with the number (Cu/II/F/26/21).

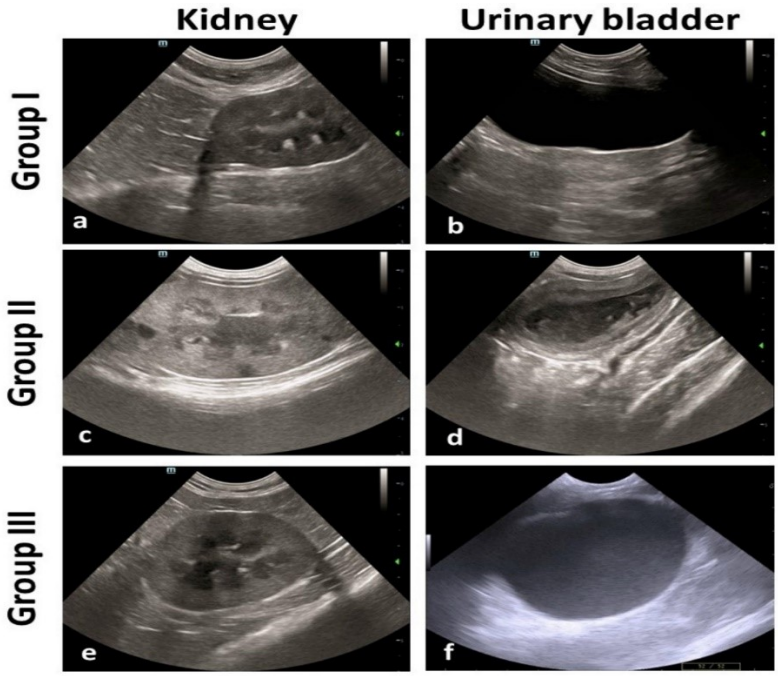

Fig. 1. Ultrasonographic findings of kidneys and urinary bladders of different groups

- **(a) Sagittal scan of kidney from group I showing normal echogenicity, size, and without any masses, cysts, or lesions.**
- **(b) Longitudinal scan of urinary bladder from group I showing normal size.**
- **(c) Sagittal scan of kidney from group II showing slight enlargement with mild loss of corticomedullary distension with multiple cysts.**
- **(d) Longitudinal scan of urinary bladder from group II showing moderate thickening of the wall with moderate echogenic multiple sediments inside the lumen.**
- **(e) Sagittal scan of kidney from group III showing normal ultrasonographic features.**
- **(f) Longitudinal scan of urinary bladders from group III showing normal ultrasonographic features**

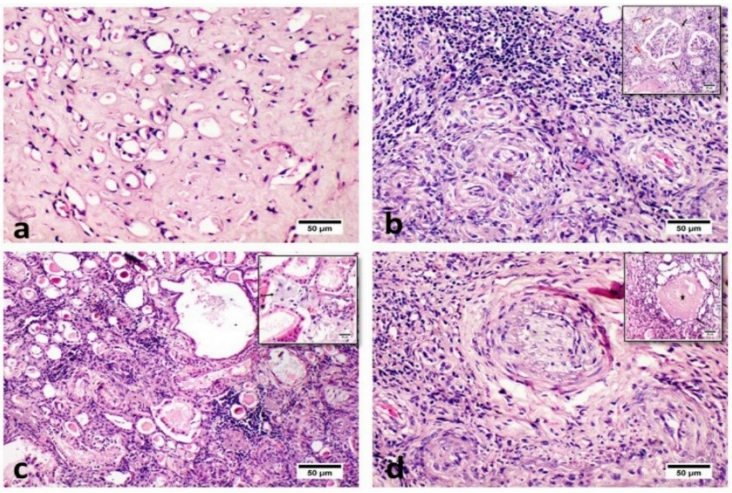

Fig. 2. Histopathological section of feline kidney (H& E) of a dead cat from group II

- **(a) Renal medulla showing extensive fibrosis with atrophied renal tubules.**
- **(b) Renal medulla showing lymphoplasmacytic cells infiltration with arteriolosclerosis and renal cortex showing periglomerular fibrosis (black arrow) and interstitial fibrosis (red arrow) in the upper right corner.**
- **(c) Renal medulla showing lymphoplasmacytic cells infiltration with cystic dilation of some renal tubules and atrophy of others filled with hyalinized proteinaceous cast and renal cortex showing needle-shaped crystals of renal tophi (arrow) were deposited in the interstitium with mononuclear cells infiltrations in the upper right corner.**
- **(d) Renal medulla showing lymphoplasmacytic cells infiltration with arteriolosclerosis and renal medulla showing ectatic renal tubules lined by flattened epithelium, producing cystic dilatation filled homogeneous proteinaceous casts (asterisk) in the upper right corner. (scale bar 50 µm)**

TABLE 1. Clinicopathological Results of Different Groups (I, II and III)

Groups / Parameters	I. Control negative	II. Control positive	III. Pre-azotemic group
Hemogram			
RBCs Count (10^6/µL)	10.33 ± 1.44[a]	5.85 ± 0.75[b]	10.10 ± 0.92[a]
Hb concentration (g/dL)	12.29 ± 1.80[a]	6.33 ± 0.91[c]	10.63 ± 1.55[b]
Hct (%)	38.07 ± 3.36[a]	17.58 ± 2.27[c]	33.53± 3.56[b]
MCV (fL)	40.44 ± 2.10[a]	34.08 ± 2.96[b]	33.95± 2.60[b]
MCHC (g/dL)	32.65 ± 1.96[a]	28.75 ± 1.98[b]	27.91 ± 2.31[c]
TLC (10^3/µL)	14.82 ± 2.62[a]	12.83 ± 1.70[b]	7.88 ± 1.09[c]
Neutrophils (10^3/µL)	11.06 ± 1.42[a]	8.22 ± 1.34[b]	4.43 ± 0.68[c]
Lymphocytes (10^3/µL)	1.93 ± 0.35[c]	3.71 ± 0.68[a]	2.52 ± 0.78[b]
Monocytes (10^3/µL)	0.63 ± 0.12[a]	0.35 ± 0.14[b]	0.34 ± 0.15[b]
Eosinophils (10^3/µL)	1.20 ± 0.33[a]	0.55 ± 0.16[b]	0.59 ± 0.16[b]
Arterial blood gas analysis (ABG)			
pH	7.42 ± 0.05[a]	7.13 ± 0.04[b]	7.35 ± 0.04[a]
PCO_2 (mmHg)	34.00 ± 1.63[a]	32.13 ± 4.12[a]	35.27 ± 1.16[a]
PO_2 (mmHg)	41.00 ± 2.94[a]	44.33 ± 3.86[a]	39.00 ± 3.56[a]
Cl^- (mEq/L)	120.67 ± 2.49[b]	137.00 ± 1.63[a]	121.67 ± 2.62[b]
HCO_3^- (mmol/L)	19.73 ± 1.03[a]	14.92 ± 0.73[b]	18.10 ± 1.49[a]
Biochemical parameters			
Traditional renal biomarkers			
Creatinine (mg/dL)	1.21 ± 0.27[b]	5.25 ± 1.29[a]	1.59 ± 0.14[b]
BUN (mg/dL)	24.68 ± 1.79[b]	95.30 ± 12.91[a]	30.57 ± 7.30[b]
Protein Profile			
Total Proteins (g/dL)	5.44 ± 0.64[a]	4.15 ± 0.60[b]	4.93 ± 0.92[a]
Albumin (g/dL)	2.14 ± 0.31[a]	2.19 ± 0.39[a]	2.02 ± 0.42[a]
Globulins (g/dL)	3.30 ± 0.67[a]	1.97 ± 0.63[b]	2.76 ± 1.09[a]
A/G ratio	0.68 ± 0.20[b]	1.35 ± 0.84[a]	0.82 ± 0.45[b]
Electrolytes and minerals			
Calcium (mg/dL)	9.52 ± 1.84[a]	9.81 ± 1.29[a]	9.12 ± 1.60[a]
Phosphorous(mg/dL)	5.88 ± 0.68[b]	8.15 ± 1.01[a]	6.29 ± 0.73[b]
Sodium (mEq/L)	147.11 ± 9.46[a]	136.78 ± 5.45[b]	148.17 ± 7.60[a]
Potassium (mEq/L)	4.82 ± 0.88[b]	6.26 ± 1.99[a]	4.42 ± 1.15[b]
Novel serum biomarkers			
SDMA (nmol/L)	40.89 ± 7.56[c]	191.19 ± 41.24[a]	145.54 ± 35.69[b]
KIM-1 (ng/ml)	4.03 ± 0.59[b]	6.47 ± 1.12[a]	5.83 ± 1.54[a]

Means ± SD with different superscript letters in the same row are significantly different at ($p \leq 0.05$).

TABLE 2. Clinicopathological Results of Group IV

Groups / Parameters	Before treatment	After treatment
Hemogram		
RBCs Count (10^6/µL)	6.01 ± 0.50^b	9.16 ± 0.56^a
Hb concentration (g/dL)	6.02 ± 0.60^b	12.12 ± 1.97^a
Hct (%)	18.34 ± 1.87^b	33.60 ± 2.24^a
MCV (fL)	34.72 ± 2.21^a	38.23 ± 2.65^a
MCHC (g/dL)	30.60 ± 0.81^a	31.71 ± 2.05^a
TLC (10^3/µL)	12.99 ± 0.90^b	15.99 ± 1.79^a
Neutrophils (10^3/µL)	8.64 ± 1.25^b	11.66 ± 2.04^a
Lymphocytes (10^3/µL)	3.72 ± 0.69^a	2.05 ± 0.56^b
Monocytes (10^3/µL)	0.36 ± 0.10^b	0.79 ± 0.20^a
Eosinophils (10^3/µL)	0.27 ± 0.08^b	1.49 ± 0.56^a
Arterial blood gas analysis (ABG)		
pH	7.28 ± 0.09^a	7.31 ± 0.01^a
PCO_2 (mmHg)	34.57 ± 3.14^a	32.50 ± 2.04^a
PO_2 (mmHg)	48.67 ± 2.62^a	47.67 ± 2.49^a
Cl^- (mEq/L)	137.33 ± 3.68^a	135.00 ± 1.63^a
HCO_3^- (mmol/L)	15.97 ± 0.25^b	19.65 ± 1.35^a
Biochemical parameters		
Traditional renal biomarkers		
Creatinine (mg/dL)	2.90 ± 0.66^a	1.73 ± 0.07^b
BUN (mg/dL)	43.65 ± 4.68^a	28.99 ± 4.20^b
Protein Profile		
Total Proteins (g/dL)	4.35 ± 0.62^b	5.50 ± 0.69^a
Albumin (g/dL)	2.42 ± 0.14^a	2.36 ± 0.27^a
Globulins (g/dL)	1.93 ± 0.70^b	3.14 ± 0.87^a
A/G ratio	1.25 ± 0.27^a	0.75 ± 0.13^b
Electrolytes and minerals		
Calcium (mg/dL)	10.93 ± 0.57^a	9.11 ± 0.33^b
Phosphorous(mg/dL)	7.38 ± 1.17^a	7.97 ± 0.83^a
Sodium (mEq/L)	135.27 ± 10.23^a	134.46 ± 4.78^a
Potassium (mEq/L)	6.56 ± 0.94^a	4.89 ± 0.32^b
Novel serum biomarkers		
SDMA (nmol/L)	121.97 ± 18.40^a	66.83 ± 9.01^b
KIM-1 (ng/ml)	7.28 ± 1.49^a	3.94 ± 0.52^b

Mean ± SD with different superscript letters in the same row are significantly different at ($p \leq 0.05$).

TABLE 3. Complete Urinalysis Results of Different Groups (I, II and III)

	I. Control negative group	II. Control positive group	III. Pre-azotemic group
Urine color			
Yellow (straw to deep)	100%	40%	100%
Red	0	60%	0
Urine odour			
Urinepherous	100%	33%	80%
Ammoniacal	0	67%	20%
Urine aspect			
Clear	100%	40%	80%
Turbid	0	60%	20%
Urine specific gravity (USG)	1.036 ± 0.02^{a}	1.020 ± 0.06^{b}	1.035 ± 0.01^{a}
Urine pH	6.71 ± 1.16^{a}	7.29 ± 0.88^{a}	7.00 ± 0.76^{a}
Dipstick-protein			
Nil	100%	13%	87%
≥ 1+	0	87%	13%
Dipstick-blood			
Nil	100%	27%	67%
≥ 1+	0	73%	33%
Dipstick-leukocyte			
Nil	100%	13%	80%
≥ 1+	0	87%	20%
Urine Sediment			
RBCs			
<5 /hpf	100%	40%	80%
>10 /hpf	0	60%	20%
Pus Cells			
<5 /hpf	100%	33%	100%
>10 /hpf	0	67%	0
Tripple phosphate			
Nil	100%	13%	80%
≥+	0	87%	20%
Calcium oxalate			
Nil	100%	27%	67%
≥+	0	73%	33%
Urinary biochemical parameters			
Creatinine (mg/ dL)	124.99 ± 3.91^{a}	67.10 ± 4.08^{c}	91.36 ± 7.22^{b}
Protein (mg/ dL)	24.74 ± 3.40^{c}	83.25 ± 6.73^{a}	33.78 ± 1.73^{b}
UPC ratio	0.20 ± 0.03^{c}	1.24 ± 0.10^{a}	0.37 ± 0.03^{b}
MAU (mg/ L)	0.57 ± 0.01^{c}	15.81 ± 1.31^{a}	1.21 ± 0.33^{b}
Sodium (mEq/L)	56.65 ± 1.67^{b}	92.43 ± 3.77^{a}	46.82 ± 9.39^{b}
FENa (%)	0.36 ± 0.11^{b}	5.45 ± 0.73^{a}	0.55 ± 0.08^{b}
uGGT (U/ L)	2.73 ± 0.79^{c}	12.77 ± 2.53^{a}	6.13 ± 0.49^{b}
Novel urinary renal biomarkers			
NAG (ng/mL)	4.74 ± 0.92^{c}	9.62 ± 2.61^{a}	7.03 ± 0.88^{b}
THP (mg/dL)	4.03 ± 0.63^{a}	1.24 ± 0.34^{b}	1.03 ± 0.16^{b}

TABLE 4. Complete Urinalysis Results of Group IV

Urine color	Before treatment	After treatment
Yellow (straw to deep)	27%	100%
Red	73%	0
Urine odour		
Urinepherous	0	100%
Ammoniacal	100%	0
Urine aspect		
Clear	13%	100%
Turbid	87%	0
Urine specific gravity (USG)	1.023 ± 0.01^{b}	1.050 ± 0.01^{a}
Urine pH	6.86 ± 0.83^{a}	6.71 ± 0.70^{a}
Dipstick-protein		
Nil	7%	67%
≥ 1+	93%	33%
Dipstick-blood		
Nil	27%	100%
≥ 1+	73%	0
Dipstick-leukocyte		
Nil	60%	87%
≥ 1+	40%	13%
Urine sediment		
RBCs		
<5 /hpf	27%	100%
>10 /hpf	73%	0
Pus cells		
<5 /hpf	0	100%
>10 /hpf	100%	0
Tripple phosphate		
Nil	13%	100%
≥+	87%	0
Calcium oxalate		
Nil	17%	100%
≥+	73%	0
Urinary biochemical parameters		
Creatinine (mg/ dL)	65.56 ± 5.87^{b}	108.73 ± 9.87^{a}
Protein (mg/ dL)	96.61 ± 13.56^{a}	43.94 ± 3.98^{b}
UPC ratio	1.48 ± 0.22^{a}	0.41 ± 0.04^{b}
MAU (mg/ L)	26.71 ± 5.94^{a}	15.51 ± 2.09^{b}
Sodium (mEq/L)	95.22 ± 23.29^{a}	92.50 ± 6.32^{a}
FENa (%)	1.25 ± 0.44^{a}	0.55 ± 0.04^{b}
uGGT (U/ L)	5.25 ± 0.87^{a}	5.74 ± 0.97^{a}
Novel urinary renal biomarkers		
NAG (ng/mL)	8.94 ± 1.89^{a}	4.02 ± 0.78^{b}
THP (mg/dL)	1.04 ± 0.39^{a}	1.10 ± 0.68^{a}

Mean ± SD of USG, pH, biochemical and Novel parameters with different superscript letters in the same row are significantly different at ($p \leq 0.05$).

References

1. Kutlu, T. and Alcigir, G. Comparison of renal lesions in cats and dogs using pathomorphological and ımmunohistochemical methods. *Biotechnic and Histochemistry*, **94**(2), 126–133 (2018). doi:10.1080/10520295.2018.1522670.
2. Brandenburger, T., Somoza, A. S., Devaux, Y. and Lorenzen, J. Noncoding RNAs in acute kidney injury. *Kidney International*, **94**(5), 870–881 (2018). doi: 10.1016/j.kint.2018.06.033.
3. Chen, H., Avital, Y., Bruchim, Y., Aroch, I. and Segev, G. Urinary heat shock protein-72: A novel marker of acute kidney injury and chronic kidney disease in cats. *Veterinary Journal*, **243**(2019), 77–81 (2019). doi: 10.1016/j.tvjl.2018.11.015.
4. Chen, H., Dunaevich, A., Apfelbaum, N., Kuzi, S., Mazaki-Tovi, M., Aroch, I. and Segev, G. Acute on chronic kidney disease in cats: Etiology, clinical and clinicopathologic findings, prognostic markers, and outcome. *Journal of Veterinary Internal Medicine*, **34**(4), 1496–1506 (2020). doi: 10.1111/jvim.15808.
5. Chew, D. J. and Schenck, P. A. *Urinalysis in the Dog and Cat*, John Wiley & Sons, Inc: USA (2023).
6. De Loor, J., Daminet, S., Smets, P., Maddens, B. and Meyer, E. Urinary biomarkers for acute kidney injury in dogs. *Journal of Veterinary Internal Medicine*, **27**(5), 998–1010 (2013). doi: 10.1111/jvim.12155.
7. Ekanayake, E. M., Gunasekara, T. D., De Silva, P. M., Jayasinghe, S., Chandana, E. P. and Jayasundara, N. Applicability of Novel Urinary Biomarkers for the Assessment of Renal Injury in Selected Occupational Groups in Sri Lanka: A Comparative Study with Conventional Markers. *International Journal of Environmental Research and Public Health*, **19**(9), 5264-5278, (2022). doi:10.3390/ijerph19095264.
8. Katayama, M., Ohata, K., Miyazaki, T., Katayama, R., Wakamatsu, N., Ohno, M., Yamashita, T., Oikawa, T., Sugaya, T. and Miyazaki, M. Renal expression and urinary excretion of liver-type fatty acid-binding protein in cats with renal disease. *Journal of Veterinary Internal Medicine*, **34**(2), 761–769 (2020). doi:10.1111/jvim.15721.
9. Hokamp, J. A. and Nabity, M. B. Renal biomarkers in domestic species. *Veterinary Clinical Pathology*, **1**, 28–56 (2016). doi: 10.1111/vcp.12333.
10. Jana, S., Mitra, P. and Roy, S. Proficient novel biomarkers guide early detection of acute kidney injury: A Review. *Diseases*, **11**(1), 8-31 (2023). doi:10.3390/diseases11010008.
11. Giapitzoglou, S., Saridomichelakis, M., Leontides, L., Kasabalis, D., Chatzis, M., Apostolidis, K., Theodoroua, K., Roumpeasa, E. and Mylonakisa, M. Evaluation of serum symmetric dimethylarginine as a biomarker of kidney disease in canine leishmaniosis due to Leishmania infantum. *Veterinary Parasitology*, **277**, 109015 (2020). doi:10.1016/j.vetpar.2019.109015.
12. McKenna, M., Pelligand, L., Elliott, J., Cotter, D. and Jepson, R. Relationship between serum iohexol clearance, serum SDMA concentration, and serum creatinine concentration in non-azotemic dogs. *Journal of Veterinary Internal Medicine*, **34**(1), 186–194 (2020). doi:10.1111/jvim.15659.
13. Ernst, R., Ogeer, J., McCrann, D., Cross, J., Strong-Townsend, M., Friis, H., Coyne, M., Clements, C., Drake, C. and Murphy, R. Comparative performance of IDEXX SDMA Test and the DLD SDMA ELISA for the measurement of SDMA in canine and feline serum. *PLoS ONE*, **13**(10), e0205030 (2018). doi:10.1371/journal.pone.0205030.
14. Dahlem, D. P., Neiger, R., Schweighauser, A., Francey, T., Yerramilli, M., Obare, E. and Steinbach, S. Plasma Symmetric Dimethylarginine Concentration in Dogs with Acute Kidney Injury and Chronic Kidney Disease. *Journal of Veterinary Internal Medicine*, **31**(3), 799–804 (2017). doi:10.1111/jvim.14694.
15. Loane, S. C., Thomson, J. M., Williams, T. L. and McCallum, K. E. Evaluation of symmetric dimethylarginine in cats with acute kidney injury and chronic kidney disease. *Journal of Veterinary Internal Medicine*, **36**(5), 1669–1676 (2022). doi:10.1111/jvim.16497.
16. Gohda, T., Kamei, N., Koshida, T., Kubota, M., Tanaka, K., Yamashita, Y., Adachi, E., Ichikawa, S., Murakoshi, M., Ueda, S. and Suzuki, Y. Circulating kidney injury molecule-1 as a biomarker of renal parameters in diabetic kidney disease. *Journal of Diabetes Investigation*, **11**(2), 435–440 (2020). doi:10.1111/jdi.13139.
17. Elkhiat, M., Abd-Elbaset Kubesy, A., Ahmed, T., Baraka, M., Abd-Elsamad Torad, F., Salem, S. and Abd-Elrahman, S. Diagnostic value of kidney injury molecule-1 in feline lower urinary tract disease. *Bioscience Research,* **16***(1), 45-53* (2019).
18. Fassett, R. G., Venuthurupalli, S. K., Gobe, G. C., Coombes, J. S., Cooper, M. A. and Hoy, W. E. Biomarkers in chronic kidney disease: A review. *Kidney International*. **80**, 806–821 (2011). doi:10.1038/ki.2011.198.
19. Aljorani, R. H., Saleh, E. S. and Al Mohammadawi, K. G. Correlation of kidney injury molecule-1 and nephrin levels in Iraqi patients with diabetic nephropathy. *Al-Rafidain Journal of Medical Sciences*, **5**, 99–104 (2023). doi:10.54133/ajms.v5i.167.
20. Waanders, F., Van Timmeren, M., Stegeman, C., Bakker, S. and Van Goor, H. Kidney injury molecule-1 in renal disease. *Journal of Pathology*,**220**, 7–16 (2010). doi:10.1002/path.2642.
21. Ichii, O. and Horino, T. MicroRNAs associated with the development of kidney diseases in humans and animals. *Journal of Toxicologic Pathology*. Japanese Society of Toxicologic Pathology **31**(1), 23–34 (2018). doi:10.1293/tox.2017-0051.

22. An, C., Akankwasa, G., Liu, J., Wang, D., Cheng, G., Zhang, J. and Qin, X. Urine markers of renal tubular injury in idiopathic membranous nephropathy: A cross sectional study. *Clinica Chimica Acta*, **492**, 7–11 (2019). doi: 10.1016/j.cca.2019.01.015.

23. Lobato, G. R., Lobato, M. R., Thomé, F. S. and Veronese, F. V. Performance of urinary kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and N-acetyl-β-D-glucosaminidase to predict chronic kidney disease progression and adverse outcomes. *Brazilian Journal of Medical and Biological Research*, **50**(5), e6106 (2017). doi:10.1590/1414431X20176106.

24. Klinkhammer, B., Djudjaj, S., Kunter, U., Palsson, R., Edvardsson, V., Wiech, T., Thorsteinsdottir M., Hardarson S., Foresto-Neto, O., Mulay, S., Moeller, M., Jahnen-Dechent, W., Floege, J., Anders, H. and Boor, P. Cellular and molecular mechanisms of kidney injury in 2,8-dihydroxyadenine nephropathy. *Journal of the American Society of Nephrology*, **31**(4), 799–816 (2020). doi:10.1681/ASN.2019080827.

25. Mohammadi-Karakani, A., Asgharzadeh-Haghighi, S., Ghazi-Khansari, M. and Hosseini, R. Determination of urinary enzymes as a marker of early renal damage in diabetic patients. *Journal of Clinical Laboratory Analysis*, **21**(6), 413–417 (2007). doi:10.1002/jcla.20212.

26. Kovarikova, S. Urinary biomarkers of renal function in dogs and cats: A review. *Veterinarni Medicina*, **60**(11), 589–602 (2015). doi:10.17221/8527-VETMED.

27. Cianciolo, R., Hokamp, J. and Nabity, M. Advances in the evaluation of canine renal disease. *Veterinary Journal*. Bailliere Tindall Ltd September **215**(2016), 21–29 (2016). doi:10.1016/j.tvjl.2016.04.012.

28. Lamb, E. J., MacKenzie, F. and Stevens, P. E. How should proteinuria be detected and measured? *Annals of Clinical Biochemistry*. **46**, 205–217 (2009). doi:10.1258/acb.2009.009007.

29. Kaneko, J. J., Harvey, J. W. and Bruss, M. L. *Clinical Biochemistry of Domestic Animals*, Eighths., Academic Press (2008).

30. Buresova, E., Stock, E., Paepe, D., Stammeleer, L., Vandermeulen, E., Smets, P., Duchateau, L., Lefebvre, H. and Daminet, S. Assessment of symmetric dimethylarginine as a biomarker of renal function in hyperthyroid cats treated with radioiodine. *Journal of Veterinary Internal Medicine*, **33**(2), 516–522 (2019). doi:10.1111/jvim.15407.

31. Bernachon, N., Fournel, S., Gatto, H., Monginoux, P. and McGahie D. Comparative palatability of five supplements designed for cats suffering from chronic renal disease. *Irish Veterinary Journal*, **67**, 10-16 (2014).

32. Mattoon, J. S., Sellon R. K. and Berry C.R. *Small Animal Diagnostic Ultrasound*, Fourth., Elsevier Inc. (2020).

33. Brooks, M. B., Harr, K. E., Seelig, D. M., Wardrop, J. K. and Weiss, D. J. *Schalm's Veterinary Hematology*, Seventh., John Wiley & Sons, Inc: USA, (2022).

34. Sant'Anna, M. C., Martins, G. F., Flaiban, K. K. M. C., Trautwein, L. G. and Martins, M. I. Protein-to-creatinine urinary in the early diagnosis of renal injury in canine pyometra. *Pesquisa Veterinaria Brasileira*, **39**(3), 186–191 (2019). doi:10.1590/1678-5150-PVB-5624.

35. Pressler, B. M. Clinical approach to advanced renal function testing in dogs and cats. *Clinics in Laboratory Medicine*, **35**(2015), 487–502 (2015). doi:10.1016/j.cll.2015.05.001.

36. Bancroft, J. and Gamble, M. *Theory and Practice of Histological Techniques*, Sixth., Elsevier: Churchill Livingstone, China (2008).

37. Hokamp, J. A., Cianciolo, R. E., Boggess, M., Lees, G. E., Benali, S. L., Kovarsky, M. and Nabity, M. B. Correlation of Urine and Serum Biomarkers with Renal Damage and Survival in Dogs with Naturally Occurring Proteinuric Chronic Kidney Disease. *Journal of Veterinary Internal Medicine*, **30**(2), 591–601 (2016). doi:10.1111/jvim.13832.

38. Elkewahy, A., Badawy, A. M., Ismail, S. F. and El-Maghraby, H. M. Ultrasonography of the urinary tract in dogs and cats: Clinical investigations and prevalence. *Benha Veterinary Medical Journal*, **45**, 54–59 (2023).

39. Elgazzar, Y. M., Ghanem, M. M., Abdel-Raof and Y. M. Helal, M. A. Clinical, haemato-biochemical and ultrasonographic diagnostic tools of different urinary tract affections in dogs. *Benha Veterinary Medical Journal*, **40**, 119–124 (2021).

40. Yan, G. Y., Chen, K. Y., Wang, H. C., Ma, T. Y. and Chen, K. S. Relationship between ultrasonographically determined renal dimensions and International Renal Interest Society stages in cats with chronic kidney disease. *Journal of Veterinary Internal Medicine*, **34**(4), 1464–1475 (2020). doi:10.1111/jvim.15814.

41. Griffin, S. Feline abdominal ultrasonography: What's normal? What's abnormal? Renal pelvis, ureters and urinary bladder. *Journal of Feline Medicine and Surgery*, **22**(9), 847–865 (2020). doi:10.1177/1098612X20941786.

42. Ostrovskyi, O. Ya. and Slivinska, L. G. Effectiveness of complex treatment of cats for chronic kidney disease. *Ukrainian Journal of Veterinary and Agricultural Sciences*, **6**(3), 56–60 (2023). doi:10.32718/ujvas6-3.11.

43. Chalhoub, S., Langston, C. E. and Eatroff, A. Anemia of Renal Disease. What It Is, What to Do and What's New. *Journal of Feline Medicine and Surgery*, **13**(9), 629–640 (2011). doi:10.1016/j.jfms.2011.07.016.

44. Gest, J., Langston, C. and Eatroff, A. Iron status of cats with chronic kidney disease. *Journal of Veterinary Internal Medicine*, **29**(6), 1488–1493 (2015). doi:10.1111/jvim.13630.

45. Roşca, M., Cristian, A. M., Huştea, L., Preda, V., Simion, R. and Codreanu, M. Case studies regarding the hematological parameters in polycystic kidney disease in cats. Scientific Works. Series C. *Veterinary Medicine*, **LXVIII** (1), 125–128 (2022).

46. Ebrahim, Z., Gameaa, S., Metwally, A. and Elsayed, M. ultrasonographic examination as a diagnostic aid in some urinary affections in cats. *Alexandria Journal of Veterinary Sciences*, **63**(1), 10-17 (2019). doi:10.5455/ajvs.26521.

47. Arai, Y., Kanda, E., Iimori, S., Naito, S., Noda, Y., Sasaki, S., Sohara, E., Okado, T., Rai, T. and Uchida, S. Low white blood cell count is independently associated with chronic kidney disease progression in the elderly: The CKD-ROUTE Study. *Clinical and Experimental Nephrology*, **22**(2), 291–298 (2018). doi:10.1007/s10157-017-1441-6.

48. Krofič Žel, M., Nemec Svete, A., Tozon, N. and Pavlin, D. Hemogram-derived inflammatory markers in cats with chronic kidney disease. *Animals*, **14**(12), 1813-1825 (2024). doi:10.3390/ani14121813.

49. Kralova-Kovarikova, S., Leva, L., Knotek, Z. and Toman, M. Changes in Lymphocyte Function and Subset Counts in Cats with Spontaneous Chronic Kidney Disease. *Veterinarni Medicina*, **61**(10), 553–559 (2016). doi:10.17221/282/2015-VETMED.

50. Dell'Osa, D. and Jaensch, S. Prevalence of clinicopathological changes in healthy middle-aged dogs and cats presenting to veterinary practices for routine procedures. *Australian Veterinary Journal*, **94**(9), 317–323 (2016). doi:10.1111/avj.12481.

51. Finch, N. Acute kidney injury in cats: part two-diagnosis of AKI. *Vet Times, 1-10* (2014).

52. Canei, D. H., Pereira, M. E., de Freitas, M. N., Arruda Trevisan, Y. P., Zorzo, C., Bortolini, J., Mendonça, A. J., Sousa, V. R. and Ferreira de Almeida, A. D. Biochemical, electrolytic, and cardiovascular evaluations in cats with urethral obstruction. *Veterinary World*, **14**(8), 2002–2008 (2021). doi: org/10.14202/vetworld.2021.2002-2008

53. Langston, C. Managing fluid and electrolyte disorders in kidney disease. *Veterinary Clinics of North America - Small Animal Practice*, ***47***(2), 471–490 (2017). doi:10.1016/j.cvsm.2016.09.011.

54. Vaden, S. L. *Clinical Small Animal Internal Medicine* (*121 Glomerular Disease)*, John Wiley & Sons, Inc. 1101–1107 (2020).

55. Donato, G., Pennisi, M. G., Persichetti, M. F., Archer, J. and Masucci, M. A Retrospective comparative evaluation of selected blood cell ratios, acute phase proteins, and leukocyte changes suggestive of inflammation in cats. *Animals*, **13**(16), 2579-2592 (2023). doi:10.3390/ani13162579.

56. Cohn, L. and Côté, E. Cote's *Clinical Veterinary Advisor: Dogs and Cats*, Elsevier, Fourth, (2019).

57. Da Costa-Val, A. P., Cambraia Veado, J. C., Ribeiro, V. M. and Muniz dos Santos, F. Evaluation of physiological biomarkers as possible predictive factors and prognosis markers of kidney injury in dogs naturally infected with *Leishmania infantum*. *Archives of Veterinary Medicine*, **16**(2), 83–102 (2023). doi:10.46784/e-avm.v16i2.327.

58. Hall, J. A., Yerramilli, M., Obare, E., Yerramilli, M. and Jewell, D. E. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. *Journal of Veterinary Internal Medicine*, **28**(6), 1676–1683 (2014). doi:10.1111/jvim.12445.

59. Wun, M. K., Broughton-Neiswanger, L. E. and Villarino, N. F. Comparison of serum SDMA and creatinine as a biomarker for the detection of meloxicam-induced kidney injury in cats. *Frontiers in Veterinary Science*, **11**, 1-8 (2024). doi:10.3389/fvets.2024.1395505.

60. Xavier Júnior, F. A., Morais, G. B., Silveira, J. A., Sampaio, T. L., Martins, A. M., Silva, I. N., Viana, D. A and Evangelista, S. A. Kidney injury molecule-1 and urinary gamma-glutamyl transferase as biomarkers of acute kidney injury in cats. *Journal of Small Animal Practice*, **63**(3), 203–210 (2022). doi:10.1111/jsap.13440.

61. Zou, C., Wang, C. and Lu, L. Advances in the study of subclinical AKI biomarkers. *Frontiers in Physiology*, **13,** 1-15 *(*2022). doi:10.3389/fphys.2022.960059.

62. Yadav, S. N., Ahmed, N., Nath, A. J., Mahanta, D. and Kalita, M. K. Urinalysis in dog and cat: A review. *Veterinary World*, **13**(10), 2133–2141 (2020). doi:10.14202/vetworld.2020.2133-2141.

63. Ribeiro, J. F., Liguori, T. T., Le Sueur, A. N., Padovani, C. R., De Arruda Monteiro, M. J. M., Melchert, A. and Guimarães-Okamoto, P. T. A transversal study of biochemical profile, urinalysis, UPC, electrolytes and blood pressure in dogs with chronic kidney disease. *Acta Scientiae Veterinariae*, **48**, 1733- 1741 (2020). doi:10.22456/1679-9216.102937.

64. Mortier, F., Daminet, S., Duchateau, L., Biscop, A. and Paepe, D. Biological variation of urinary protein: Creatinine ratio and urine specific gravity in cats. *Journal of Veterinary Internal Medicine*, **37**(6), 2261–2268 (2023). doi:10.1111/jvim.16881.

65. Gori, E., Pierini, A., Lippi, I., Boffa, N., Perondi, F. and Marchetti, V. Urinalysis and urinary GGT-to-urinary creatinine ratio in dogs with acute pancreatitis. *Veterinary Sciences*, **6**(1), 27-33 (2019). doi:10.3390/VETSCI6010027.

66. Fidalgo, M. A., Leal, R. O. and Duarte-Correia, J. H. Urinary protein/creatinine ratio in feline medicine: reasons to perform it and its role in clinical practice-A Retrospective study. *Animals*, **12**(12), 1575-1583 (2022). doi:10.3390/ani12121575.

67. Elliott, J., Grauer, G. F. and Westropp, J. L. *BSAVA Manual of Canine and Feline Nephrology and Urology*, Third., (2017).

68. Williams, T. and Archer, J. Evaluation of urinary biomarkers for azotaemic chronic kidney disease in cats. *Journal of Small Animal Practice*, **57**(3), 122–129 (2016). doi:10.1111/jsap.12439.

69. Chakrabarti, S., Syme, H. and Elliott, J. Clinicopathological variables predicting progression of azotemia in cats with chronic kidney disease. *Journal of Veterinary Internal Medicine*, **26**(2), 275–281 (2012). doi:10.1111/j.1939-1676.2011.00874.x.

70. Sayed, S. Y., Salem, S. I., Abdallah, A. N., Khalil, G. M. and Mohammed, F. F. Clinicopathological studies on the use of laser-activated adipose-derived stromal vascular fraction in treatment of streptozotocin-induced diabetes in rats. *Comparative Clinical Pathology*, **28**(5), 1515–1526 (2019). doi:10.1007/s00580-019-03008-8.

71. Thakar, S. and Paller, M. S. Sodium Metabolism in Chronic Kidney Disease. In *Chronic Renal Disease*, 633–641 (2020). doi:10.1016/B978-0-12-815876-0.00039-5.

72. Lefebvre, H. P., Dossin, O., Trumel, C. and Braun, J. P. Fractional excretion tests: A critical review of methods and applications in domestic animals. *Veterinary Clinical Pathology*, **37**(1), 4–20 (2008). doi:10.1111/j.1939-165X.2008.00010.x.

73. Mircean, M., Codea, A. R., Biris, A., Beres, C., Nicolae, M., Popovici, C. and Neagu, D. Urinary N-acetyl-B-D-glucosaminidase index evaluation in cats with naturally acquired feline immunodeficiency virus infection. *Revista Romana de Medicina Veterinara*, **33**(*4)*, 55–58 (2023).

74. Crisi, P. E., Dondi, F., De Luca, E., Di Tommaso, M., Vasylyeva, K., Ferlizza, E., Savini, G., Luciani, A., Malatesta, D., Lorusso, A. and Boari, A. Early renal involvement in cats with natural feline morbillivirus infection. *Animals*, **10**(5), 828- 842 (2020). doi:10.3390/ani10050828.

75. Ferlizza, E., Isani, G., Dondi, F., Andreani, G., Vasylyeva, K., Bellei, E., Almeidad, A. and Matzapetakise, M. Urinary proteome and metabolome in dogs (Canis lupus familiaris): The effect of chronic kidney disease. *Journal of Proteomics*, **222**, 103795 *(*2020). doi:10.1016/j.jprot.2020.103795.

76. Afsar, B., Afsar, R. E., Dagel, T., Kaya, E., Erus, S., Ortiz, A., Covic, A. and Kanbay, M. Capillary rarefaction from the kidney point of view. *Clinical Kidney Journal*, **11**(3), 295–301 (2018). doi:10.1093/ckj/sfx133.

77. Paschall, R. E., Quimby, J. M., Cianciolo, R. E., McLeland, S. M., Lunn, K. F. and Elliott, J. Assessment of peritubular capillary rarefaction in kidneys of cats with chronic kidney disease. *Journal of Veterinary Internal Medicine,* **37***,* 556-566 (2023). doi:10.1111/jvim.16656.

78. Kida, Y. Peritubular capillary rarefaction: An underappreciated regulator of CKD progression. *International Journal of Molecular Sciences*. **21**, 8255-8278 (2020). doi:10.3390/ijms21218255.

79. McLeland, S. M., Cianciolo, R. E., Duncan, C. G. and Quimby, J. M. A Comparison of biochemical and histopathologic staging in cats with chronic kidney disease. *Veterinary Pathology*, **52**(3), 524–534 (2015). doi:10.1177/0300985814561095.

80. Nangaku, M. Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. *Journal of the American Society of Nephrology*, **17**(1), 17–25 (2006). doi:10.1681/ASN.2005070757.

أمراض الكلى في القطط : الدلالات الحيوية الجديدة مقابل التقليدية

غادة محمد خليل[1]، خالد محمد أحمد مهران[1]، محمد السعيد[2] وشيماء إسماعيل سالم[1]

[1] قسم الباثولوجيا الإكلينيكية - كلية الطب البيطري - جامعة القاهرة - مصر.

[2] قسم الأمراض الباطنة والمعدية - كلية الطب البيطري - جامعة القاهرة – مصر.

الملخص

تُعتبر أمراض الكلى من الاضطرابات الشائعة لدى القطط، وتُعتبر من الأسباب الرئيسية للوفاة، كما أنها تُشكل تحديًا تشخيصيًا بسبب نقص العلامات المبكرة للتلف. تتمتع الدلالات الحيوية التقليدية (مثل مستوى الكرياتينين في السيرم وتركيز نيتروجين اليوريا في الدم) بحساسية تشخيصية منخفضة، وبالتالي، تتحول الحالات الحادة إلى مزمنة. هدفت هذه الدراسة إلى تقييم توضيح النتائج الباثولوجية الإكلينيكية، والموجات فوق الصوتية، والنتائج الباثولوجية للقطط المصابة بأمراض الكلى، وأداء الدلالات الحيوية الجديدة لتلف الكلى مثل SDMA وKIM-1 وNAG وTHP ومقارنتها بالدلالات الحيوية التقليدية لوظيفة الكلى ودورها في متابعة الحالات المعالجة. تم تقسيم 86 قطة إلى 4 مجموعات بناءً على الأعراض السريرية، وتركيزات مستوى الكرياتينين في الدم (sCr) وكثافة البول النوعية (USG) كما يلي: I.المجموعة الضابطة للحيوانات السليمة ظاهريا (15 قطة) ، II.المجموعة الضابطة للحيوانات المريضة (22 قطة مصابة بأمراض الكلى)، III.المجموعة ما قبل الأزوتيميا (31 قطة)، و. IVالمجموعة المعالجة (18 قطة). تم جمع عينات الدم والبول للهيماتولوجي، غازات الدم (ABG)، كيمياء الدم والتحليل الروتيني للبول، بالإضافة إلى تحليل الدلالات الحيوية الجديدة في السيرم والبول. أظهرت نتائج المجموعة الثانية والثالثة أنيميا نقص الحديد والنقص المعنوي لكريات الدم البيضاء، زيادة تركيز كلا من SDMA وKIM-1 في السيرم ونشاط NAGفي البول في حين لوحظ انخفاض كبير في تركيز THP في البول. أظهرت المجموعة IV بعد العلاج تحسنا ملحوظا في كل النتائج فيما عدا تركيز.THP وقد أظهرت نتائج الدراسة أن تركيز كلا من SDMAوKIM-1 ونشاط NAG يمكن أن تكون دلالات حيوية فعالة للإكتشاف المبكر لتلف الكلى وتحسن وظائفها.

الكلمات الدالة: SDMA ، KIM-1 ، NAG ، THP ، تحليل البول.