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Diagnostic Value of Kidney Injury Molecule- 1 in Feline Lower Urinary Tract Disease

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This study was carried out to investigate the diagnostic value of kidney injury molecule-1(KIM-1) in feline lower urinary tract disease (FLUTD). Forty seven Persian cats involved in this study divided into control group (9 cats) and FLUTD cases (31 obstructive and 7 non obstructive). Blood serum and urine samples were examined for determination of serum KIM-1, creatinine, BUN, sodium, potassium and urine KIM-1, physical, chemical and microscopical examination. Ultrasonography and histopathology of kidney and bladder were performed. Level of serum KIM-1 in cases of obstructive FLUTD was significantly increased about 20 fold than control group while in non-obstructive cats there was significant 2 fold increase. Kidney injury molecule 1 could be used as early diagnostic and prognostic test in suspected acute kidney injuries.

Keywords: kidney injury molecule 1- feline lower urinary tract disease- obstructive - ultrasonography - histopathology.

INTRODUCTION

Feline lower urinary tract disease (FLUTD) is a general term describe a multifactorial problem with clinical signs including dysuria, hematuria, pollakiuria, and with or without urinary obstruction (Lund et al., 2013 and Dorsch et al., 2014). It is one of the most common causes for cat admissions to veterinary hospitals (4 -10% of all feline admissions are regarded to FLUTD). FLUTD has a high recurrence rate ranges between 30% to 70% and mortality rate 6- 36 % (Grauer, 2014).

Obstructive uropathy is a mutual complication of FLUTD which is considered an urologic emergency condition (Schmeltzer and Norsworthy, 2012 and Hall et al., 2015) .

Acute kidney injury in cats is infrequently diagnosed, that is regarded to limitations of diagnostic aids and/or the subclinical nature of such disease. Cases with apparent or incomplete renal recovery may progress to chronic kidney disease with negative impact on health and quality of live (Reynolds and Lefebvre, 2013 and Bland et al., 2014).

Serum creatinine concentration (SCC) is considered the most routinely applied test for determination of kidney function and diagnosis of renal diseases .It has defective limitations including low sensitivity as it only exceeds the normal range when approximately 75% of renal tissue is deteriorated and imperfect specificity due to endogenous synthesis of creatinine by muscles making muscle mass, breed and sex a influencing factors of SCC (Bland et al., 2014; Hall et al., 2014).

Kidney injury molecule I (KIM-1) is a renal tubular transmembrane glycoprotein act in cell-to-cell or cell-to- matrix adhesion also recognized as hepatitis A virus cell receptor 1 and T cell immunoglobulin 1, it was hypothesized that its level increase with toxic and ischemic kidney injury in rodents and human (Bland et al., 2014).

Feline kidney injury molecule -1 is conserved across species glycoprotein which is renal expressed then shed into cat urine with cases suffering from acute kidney injury. it is expressed in particular segments of the renal tubules. Urine KIM-1 immunoassay is conceivably an accurate and sensitive indicator of tubular injury.(Bland et al., 2017, 2014)

The present study is aimed to monitor changes of kidney injury molecule 1 in both serum and urine as a novel diagnostic method in comparison with other routine diagnostic procedures of acute kidney injury accompaniment to FLUTD.

MATERIALS AND METHODS

Animals

A total number of 47 cats were studied in the present research including 38 cats suffering from feline lower urinary tract disease, (7 non-obstructive and 31 obstructive FLUTD) and 9 apparently healthy normal cats, admitted to the clinic of the Faculty of Veterinary Medicine, Cairo university. Each of those cats exposed to complete physical, clinical, ultrasonography and laboratory examinations of urinary system.

During study period, 2 cats deceased. These cases under went post mortem examination and samples were taken for histopathological examination. Both of the kidney and the urinary bladder of each cat was carefully dissected out and fixed in 10% buffered neutral formalin. Formalin fixed specimens were routinely dehydrated in graded series of alcohol and cleared in xylol then finally embedded in paraffin. Paraffin blocks were sectioned at 4-5 um thickness and routinely stained with H&E (Bancroft and Gamble, 2008).

Blood samples

blood samples were withdrawn in serum separator tube, serum samples were preserved in -20 and used for determination of BUN, creatinine, sodium and potassium using specific test kits (spectrum diagnostic-Egypt) and kidney injury molecule 1(KIM1) determined using specific feline ELISA kit (catalogue No# MBS104549, Mybiosource, USA).

Urine Samples

The Urine samples were collected from all groups of cats by gentle manipulation of urinary bladder, catheterization or cystocentesis and examined within 30 minutes. Urine samples were exposed to complete physical, chemical and microscopical examination according to (Grauer 2014). Urine pH was determined using pH meter. Urine specific gravity was determined using hand refractometer. KMI-1 was determined using Rat KIM1 (cat no#MBS265649, My biosource, USA).

Ultrasonography

Ultrasonographic examination of the kidneys was performed using 5 and 7.5 MHz sector transducer (Mindray dp10, China) according to the method described by Nyland (2014).

statistical analysis

Analysis of data was performed using SPSS statistical program Version 20 using ANOVA and expressed as mean±SE at.

RESULTS

The serum biochemistry of affected cases (table1) showed serum kidney injury molecule 1 had significant high increase(about 20 folds) in obstructive type of feline lower urinary tract disease when compared with apparently healthy cat serum, while in serum of cats suffering from non-obstructive FLUTD there was significant low increase of KIM-1(about 2 folds). These changes may be regarded to the post renal acute renal injury which induced by urinary tract obstruction occur in obstructive FLUTD. Changes were less significant in non-obstructive type as the effect of obstruction of urinary tract is more injurious on renal tissue than the effect of slight dehydration, dry food feeding and lower urinary tract inflammation associated with non-obstructive type. Scanty papers are available about KIM1 response in FLUTD affected cats. these observations related to increased level of KIM-1caused by acute nephropathy is in concur with the work applied on rat and human reported by (van Timmeren et al., 2006; Sabbisetti et al., 2014 and Jin et al., 2017).

Table 1: Serum biochemistry of healthy and FLUTD cases:

Parameters	Apparently healthy	Obstructive FLUTD	Non obstructive FLUTD
KIM-1 (pg/ml)	19.9±1.6[a]	349.1±53.47[b]	33.24±4.38[c]
Creatinine (mg/dl)	1.9 ± 0.09[a]	15.64±1.25[b]	2.0±0.13[a]
BUN (mg/dl)	16.3±0.85[a]	117.92±2.22[b]	18.2±1.2[a]
Sodium (mmol/L)	154.2±1.4[a]	142.46±5.8[a]	156.8±1.8[a]
Potassium (mmol/L)	6.05±0.56[a]	9.8±0.8[b]	5.3±0.1[a]

Table 2: urine analysis finding of healthy and FLUTD cases:

Parameters	Apparently healthy	Obstructive FLUTD	Non Obstructive FLUTD
Color	Amber yellow to deep yellow	Light red to dark brown	Pale yellow to brown
Aspect	Clear	Turbid	Clear to turbid
Odor	urineferous	offensive	Offensive
Specific gravity	1.058±0.001[a]	1.026±0.002[b]	1.063±0.005[a]
pH	7.23±0.1[a]	7.3±0.06 [a]	7.47±0.15 [a]
Glucose	Nil	Nil to 3+	Nil
Protein	Nil	1+ to 3+	Trace to 3+
Keton bodies	Nil	Nil	Nil
Bilirubin	Nil	Nil	Nil
Urobilinogen	Nil	Nil	Nil
Nitrite	Nil	Nil	Nil
RBCs (cell/hpf)	Nil	50 to > 100	0-100
WBCs (cell/hpf)	Nil	5 - 20	3-8
Crystals	0 to traces	Struvite or oxalate	Struvite and/or oxalate
Casts	Nil	+ve	Nil to +ve
Epithelial cells	Nil	5-15	Nil to 4

findings related to significant high increase in serum creatinine ,blood urea nitrogen and serum potassium level in cases of obstructive FLUTD is regarded to post renal obstructive azotemia and this fit with observations of(Chew, DiBartola and Schenck, 2011; Segev et al., 2011 and Grauer, 2014)Urinalysis of cases affected with obstructive FLUTD showed significant hematuria in all cases which range from light degree to severe bleeding in urine while in non obstructive cases range from light turbidity in aspect to slight hematuria. Specific gravity of obstructive cats urine showed significant decrease from control cases while cats with non obstructive cases showed no significant increase. Both obstructive and non obstructive cases showed proteinuria with different degree however severity of proteinuria was increased and highly remarkable in obstructive cases. 15 case of obstructive FLUTD found suffering from glucosuria which is not found in non-obstructive cases.Crystalluria was evident in both conditions (19 of obstructive FLUTD and 7 of non obstructive type) which may be regarded to masking of crystals with crowds of rbcs and wbcs in obstructive conditions. Struvite crystals found in 12 obstructive FLUTD affected cats (from 31 case) and 3 non obstructed cats (from 7 cases). Calcium oxalate crystals seen in 5 obstructive cases and 2 non obstructive cats. Mixed crystals reported in 2 obstructive and 2 non obstructive cases. Casts was evident in obstructive cases with 5 cases with granular type and 2 erythrocytic while only 1 case in non obstructive type found with erythrocytic cast.

Hematuria, pyuria and crystalluria observed in obstructive FLUTD match with observations reported by (Segev et al., 2011) related to cystitis and urethritis . Specific gravity showed significant decrease in obstructed cats which may be regarded to kidney injury associated with post-renal obstructions concur with that reported by (Westropp et al*.,* 2005, Squires 2007 and Westropp and Buffington 2010).

KIM-1 showed significant increase in urine of obstructive cases and non obstructive cases. the obstructive cases showed no significant increase when compared to non obstructive ones. the two fold increase in urinary KIM1concurrent with obstructive cases may be explained on bases of renal tubular damage associated with post renal acute renal injury. significant little increase found in non-obstructive cases may be regarded to little damage caused by dehydration associated with non-obstructive cases. those observations are parallel to that seen by in concur with the work applied on rat and human reported by (van Timmeren et al., 2006; Sabbisetti et al., 2014 and Jin et al., 2017) in human and rats and what found by (Bland et al., 2017) in cats.

Ultrasonographic examination of obstructed tom cats showed Increased cortical echogenicity with preserved well differentiation between cortical and medullary echogenicity and increased renal size which indicate acute renal insufficiency was diagnosed in 19 tom cats. Increased cortical echogenicity with decreased differentiation between cortical and medullary echogenicity which regarded to arousal of chronic renal insufficiency was presented in 2 cases with history of recurrent obstructive FLUTD. Hydronephrosis was seen in 3 affected cats while dilated renal pelvis (without deformity in renal medulla) was found in 7 cases. Changes in urinary bladder including presence of hyperechoic urinary bladder sediment with over distension of bladder were the prominent finding in most cases. In 3 obstructed cats there is only distension of urinary bladder with anechoic urine without any other changes.

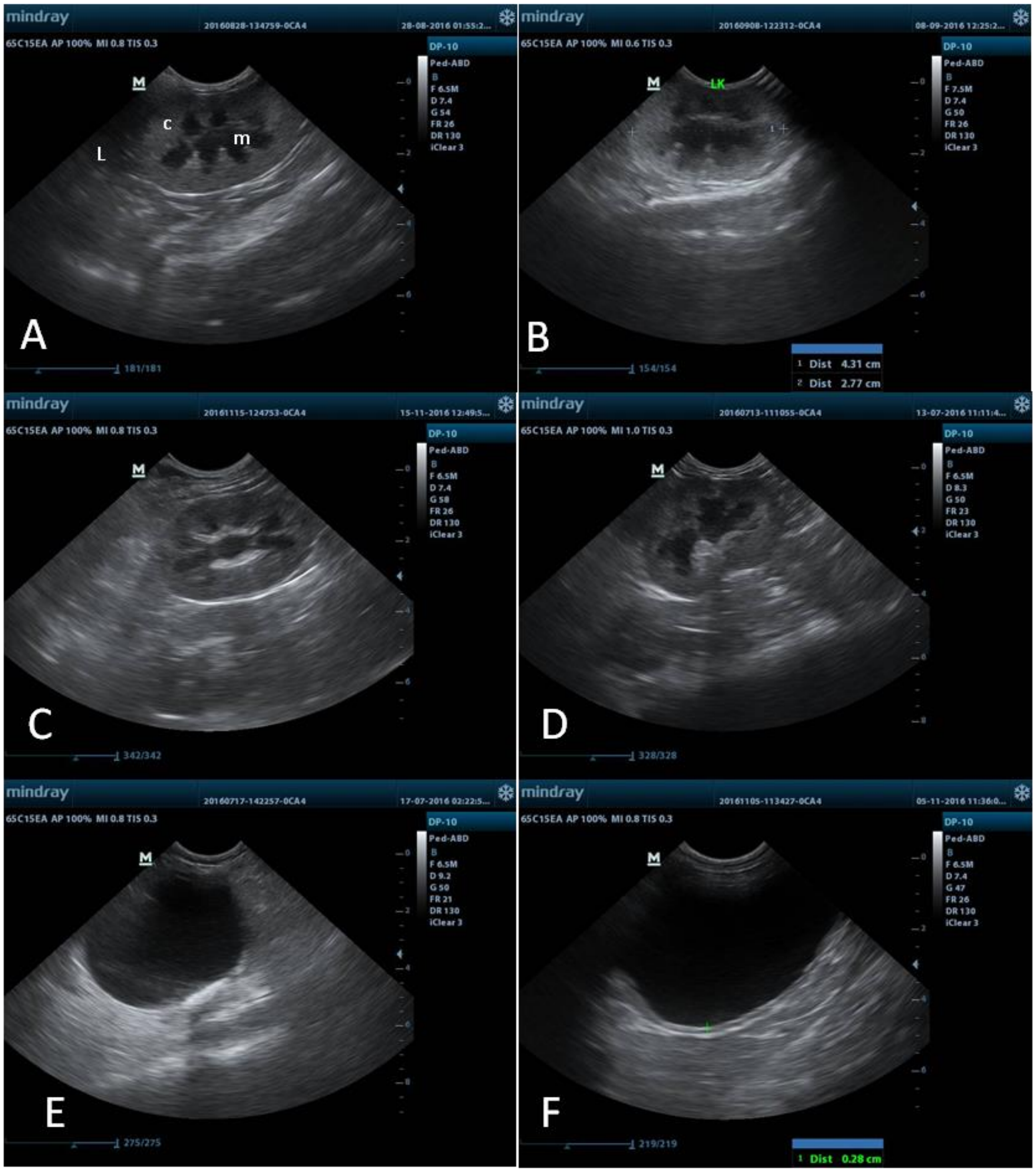
In 7 cats suffered from non-obstructive FLUTD chronic cystitis with identified increased urinary bladder wall thickness and presence of cystic stone were presented in 3 cases (2 queens and 1 tom cat). Chronic cystitis with increased bladder wall thickness was identified in 3 female cats. One tom cat showed negative ultrasonographic changes. In only 3 cases of those cats were suffering from acute renal insufficiency changes.

Acute renal insufficiency evidenced as a result of congestion and dilatation of cortical blood vessels and glomeruli. Chronic nephritis showed increased corticomedullary echogenicity due to increased interstitial connective tissue as most cases of chronic nephritis is mainly interstitial type. Hydronephrosis and dilated renal pelvis reflect invasion of renal pelvis to renal parenchyma due to recurrent or chronic obstruction. These observations found to be in concur with (debruyn et al., 2012 and mattoon and nyland, 2014).

Table 3. levels of KIM-1in urine of healthy and FLUTD cases

Parameters	Apparently healthy	Obstructive FLUTD	Non Obstructive FLUTD
KIM-1	**53.01±2.9[a]**	**100.4±8.1[b]**	**74.58±7.49[b]**

Figure: 1 illustration of ultrasonography of kidney and urinary bladder in obstructive FLUTD cases:



(A) sagittal scan of right kidney showing increased cortical echogenicity (c) over the neighboring hepatic lobe (L) with maintained corticomedullary differentiating ability (m).

(B) sagittal scan of right kidney showing increased cortical echogenicity more than the neighboring hepatic lobe with decrease in differentiation between cortical and medullary echogenicity.

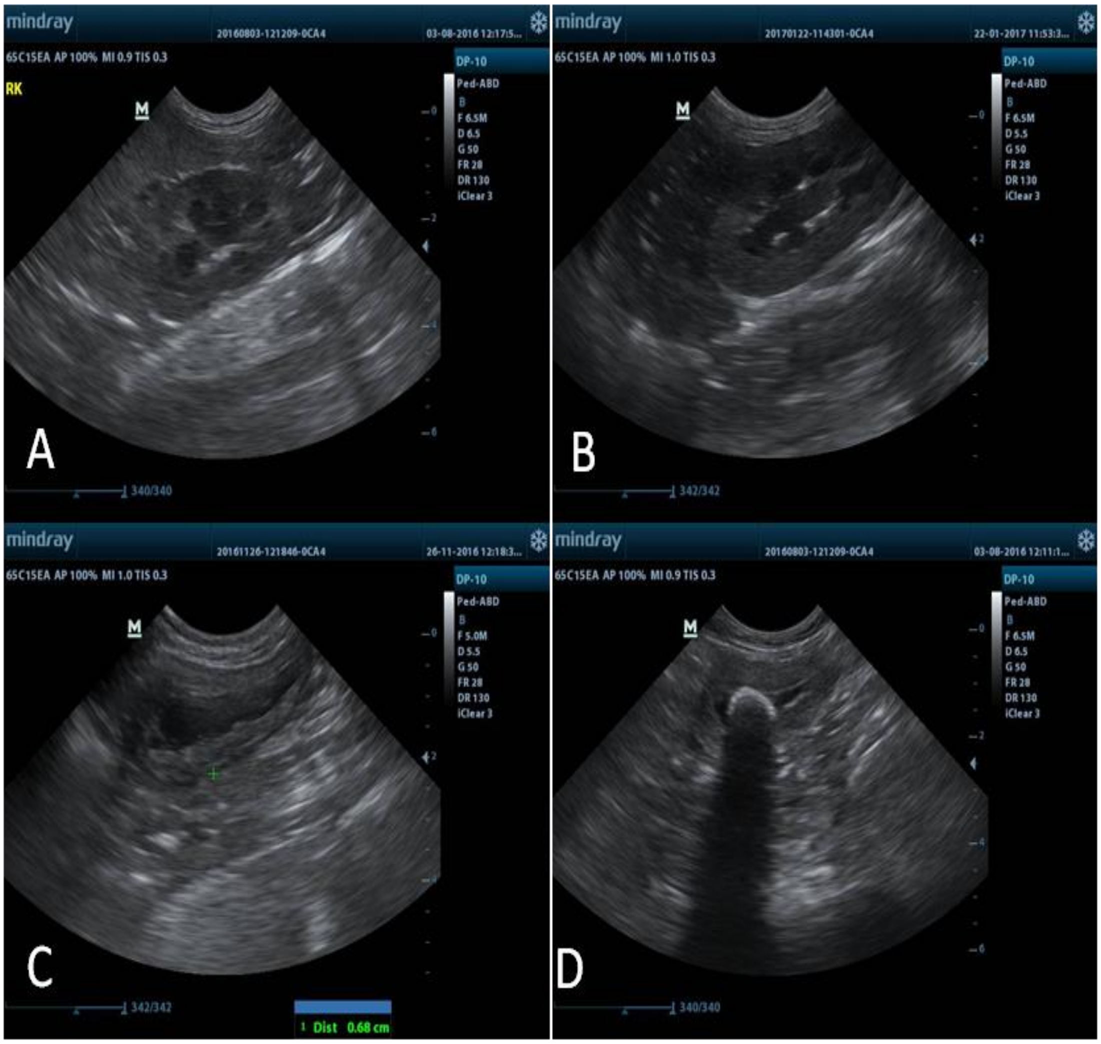
(C)sagittal scan of left kidney showing dilated renal pelvis without deformity in medullary structure.

(D) longitudinal scan of left kidney showing increase in medullary size in extent of cortical size with distortion in medullary architecture.

(E) longitudinal scan of urinary bladder showing distension in urinary bladder with distal hyperechoic sediment with distal shadowing.

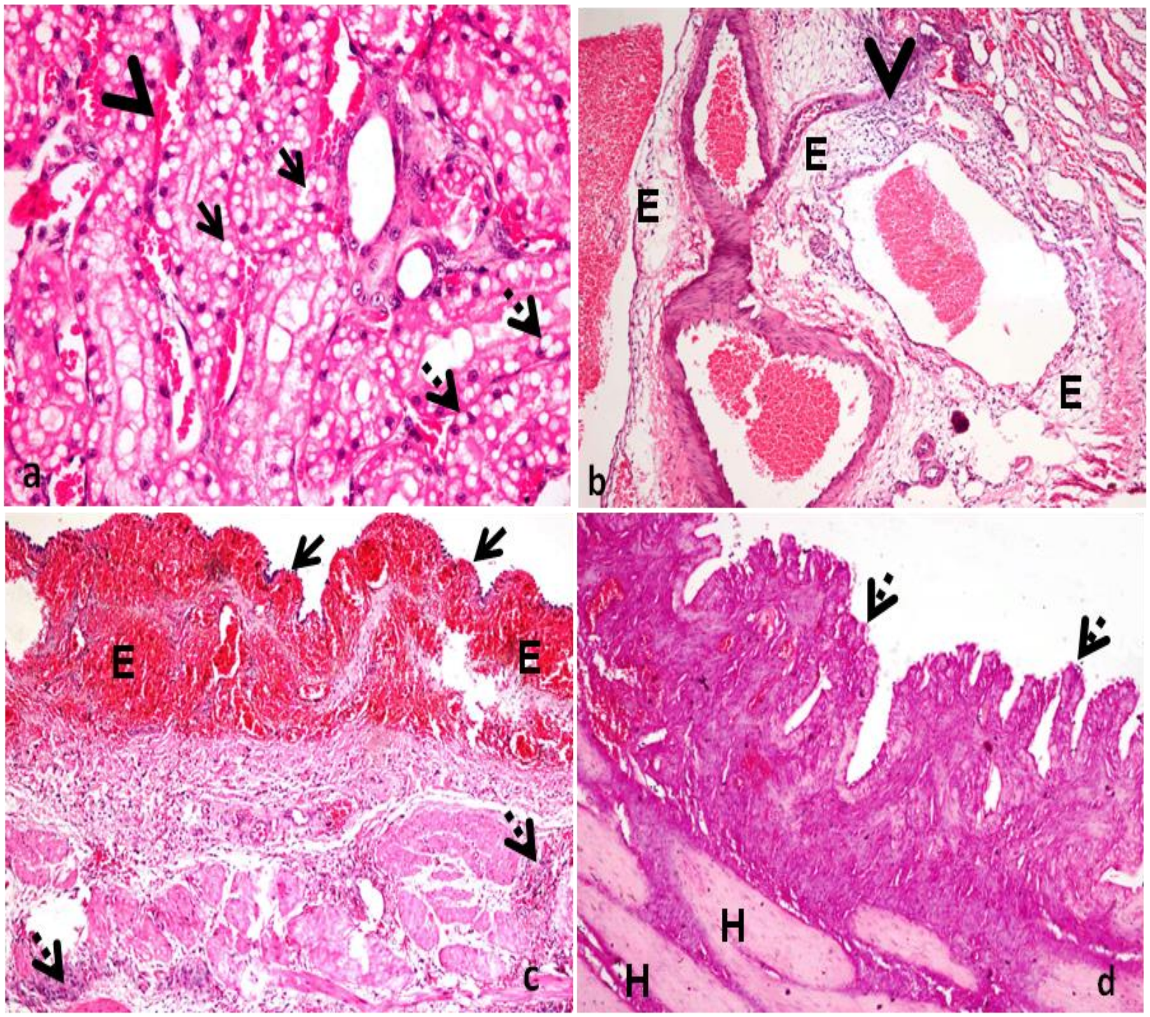
(F) longitudinal scan of urinary bladder showing distension in urinary bladder with anechoic urine.

Figure. 2 illustration of ultrasonography of kidney and urinary bladder in non obstructive FLUTD cases:



(A) sagittal scan of right kidney in a queen showing cortical echogenicity lower than neighboring hepatic lope with well differentiated corticomedullary echogenicity.
(B) sagittal scan of right kidney in female cat showing increased cortical echogenicity higher than the neighboring hepatic lope.
(C)longitudinal scan of urinary bladder in tom cat with increased wall thickness(0.68cm).
(D longitudinal scan of urinary bladder in a queen with increased wall thickness and presence of hyperechoic cystic stone with distal shadowing.

Figure. 3 illustration of histopathology of kidney and urinary bladder in obstructive FLUTD cases:



a: Kidney of cat suffered from OFLUTD showing diffuse vacuolar degeneration (arrow) of the renal tubular epithelial linings and signet ring appearance (dashed arrow) of many epithelial cells as well as intertubular hemorrhages (arrow head). **(H&E, X400).**

b: Lower medulla of cat suffered from OFLUTD showing vascular dilatation with marked vasculitis, notice edema (E) and the inflammatory cells infiltration (arrow head) in the blood vessel wall. **(H&E, X400).**

c: Urinary bladder of cat suffered from OFLUTD showing; hemorrhagic (H) lamina propria and its vascular congestion, marked necrosis and loss of the urothelium (arrow) as well as congestion and few mononuclear inflammatory cells infiltration (dashed arrow) in the muscular layer. **(H&E, X200)**

d: Urinary bladder of cat suffered from OFLUTD with ruptured urinary bladder showing necrosis and desquamation of the urothelium (dashed arrow) and hyalinization (H) of the muscle fibers of the muscular layer. **(H&E, X200).**

CONCLUSION

In FLUTD ultrasonography coupled with histopathology, traditional serum and urine analysis are considered a preliminary step for diagnosing level of kidney injury, However KIM-1 showed significant superiority in detecting level of kidney injury even in early stages compared with routine serum bio Test (creatinine and BUN). we can recommend kidney injury molecule 1 to be used as early diagnostic and prognostic test in suspected acute kidney injuries.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest. This work was partially funded by Cairo University as a part of academic PHD thesis

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AUTHOR CONTRIBUTIONS

Elkhiat MA performed collection and analysis of serum and urine samples and applied laboratory analysis. Kubesy AA, designed the study and reviewed the manuscript. Baraka TA and Shaymaa IS helped in analytical analysis. Torad FA reviewed ultrasonography reports. Sahar SA reviewed histopathology reports. Manuscript reviewed by all authors.

REFERENCES

Bland SK, Côté O, Clark ME, Delay J and Bienzle D, 2014. Characterization of Kidney Injury Molecule-1 in Cats. J. Vet. Intern. Med. 28, 1454–1464. https://doi.org/10.1111/jvim.12428

Bland SK, Schmiedt CW, Clark ME, DeLay J and Bienzle D, 2017. Expression of Kidney Injury Molecule-1 in Healthy and Diseased Feline Kidney Tissue. Vet. Pathol. 54, 490–510. https://doi.org/10.1177/0300985817690213

Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. (2008). Churchill Livingstone, China: Elsevier.

Chew D, DiBartola S, Schenck P, 2011. Canine and Feline Nephrology and Urology,2nd edn, Philadelphia: Saunders, (2011). https://doi.org/10.1016/C2009-0-53834-2

Debruyn K, Haers H, Combes A, Paepe D, Peremans K, Vanderperren K, Saunders JH, 2012. Ultrasonography of the feline kidney: Technique, anatomy and changes associated with disease. J. Feline Med. Surg. 14, 794–803. https://doi.org/10.1177/1098612X12464461

Dorsch R, Remer C, Sauter-Louis C, Hartmann K, 2014. Feline lower urinary tract disease in a German cat population: A retrospective analysis of demographic data, causes and clinical signs. Tierarztl. Prax. Ausgabe K Kleintiere - Heimtiere 42, 231–239.

Grauer GF. Urinary Tract Disorders. In: Nelson, R., Couto, G. eds.Small Animal Internal Medicine. 5th edn.Mosby, 2014.

Hall JA, Yerramilli M, Obare E, Yerramilli M, Jewell DE, 2014. Comparison of Serum Concentrations of Symmetric Dimethylarginine and Creatinine as Kidney Function Biomarkers in Cats with Chronic Kidney Disease. J. Vet. Intern. Med. 28, 1676–1683. https://doi.org/10.1111/jvim.12445

Hall J, Hall K, Powell LL, Lulich J, 2015. Outcome of male cats managed for urethral obstruction with decompressive cystocentesis and urinary catheterization: 47 cats (2009-2012). J. Vet. Emerg. Crit. Care 25, 256–262. https://doi.org/10.1111/vec.12254

Jin Y., Shao X, Sun B, Miao C, Li Z, Shi, Y, 2017. Urinary kidney injury molecule-1 as an early diagnostic biomarker of obstructive acute kidney injury and development of a rapid detection method. Mol. Med. Rep. 15, 1229–1235. https://doi.org/10.3892/mmr.2017.6103

Lund HS, Krontveit RI, Halvorsen I, Eggertsdóttir AV, 2013. Evaluation of urinalyses from untreated adult cats with lower urinary tract disease and healthy control cats: Predictive abilities and clinical relevance. J. Feline Med. Surg. 15, 1086–1097. https://doi.org/10.1177/1098612X13492739

Nyland TG, Widmer WR, Mattoon JS. Urinary tract. In: Mattoon JS, Nyland TG, eds. Small Animal Diagnostic Ultrasound. 3rd ed. St. Louis, MO: Elsevier Saunders; 2014:557-607.

Reynolds BS, Lefebvre HP, 2013. Feline CKD: Pathophysiology and risk factors - what do we know? J. Feline Med. Surg. 15, 3–14. https://doi.org/10.1177/1098612X13495234

Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, Briskin R, De Jager PL., Ngo TT, Radlinski M, Dear JW, Park KB, Betensky R, Krolewski AS, Bonventre J V, 2014. Blood Kidney Injury Molecule-1 Is a Biomarker of Acute and Chronic Kidney Injury and Predicts Progression to ESRD in Type I Diabetes. J. Am. Soc. Nephrol. 25, 2177–2186. https://doi.org/10.1681/ASN.2013070758

Schmeltzer LE, Norsworthy GD, 2012. urinary tract diseases, in: Schmeltzer, L.E., Norsworthy, G.D. (Eds.), Nursing the Feline Patient. Wiley-Blackwell, pp. 213–218.

Segev G, Livne H, Ranen E, Lavy E, 2011. Urethral obstruction in cats: Predisposing factors, clinical, clinicopathological characteristics and prognosis. J. Feline Med. Surg. 13, 101–108. https://doi.org/10.1016/j.jfms.2010.10.006

Squires RA. Laboratory evaluation of renal disorders. In:Villiers E and Blackwood L, eds.BSAVA manual of canine and feline clinical pathology. 2nd edn. British Small Animal Veterinary Association (2007).

van Timmeren MM, Bakker SJL, Vaidya VS, Bailly V, Schuurs T , Damman J, Stegeman C, Bonventre, J. V, van Goor, H., 2006. Tubular kidney injury molecule-1 in protein-overload nephropathy. Am. J. Physiol. Renal Physiol. 291, F456–F464. https://doi.org/10.1152/ajprenal.00403.2005

Westropp JL, Buffington CA, Chew DJ. Feline lower urinary tract diseases. In: Ettinger SJ, Feldman EC, eds. Textbook of veterinary internal medicine. 6th edn. Philadelphia: Saunders, (2005): 1828e50.

Westropp JL, Buffington CA. lower urinary tract disorders in cats. In: Ettinger SJ, Feldman EC, eds. Textbook of veterinary internal medicine. 7th edn. Philadelphia: Saunders, (2010): 2069e86.