

# **A STUDY ON EARLY DIAGNOSIS OF ACUTE KIDNEY DISEASE AND ITS THERAPEUTIC MANAGEMENT IN DOGS**

**Thesis**

**Submitted to Guru Angad Dev Veterinary and Animal Sciences University  
in partial fulfillment of the requirements for the degree of**

**MASTER OF VETERINARY SCIENCE  
in  
VETERINARY MEDICINE  
(Minor Subject: Veterinary Surgery and Radiology)**

**By**

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(L-2019-V-54-M)**



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## **CERTIFICATE – I**

This is to certify that the thesis entitled, “**A STUDY ON EARLY DIAGNOSIS OF ACUTE KIDNEY DISEASE AND ITS THERAPEUTIC MANAGEMENT IN DOGS**” submitted for the degree of **M.V.Sc.**, in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Surgery and Radiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Kuljeet Singh Dhaliwal (L-2019-V-54-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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## **CERTIFICATE-II**

This is to certify that the thesis entitled, “**A STUDY ON EARLY DIAGNOSIS OF ACUTE KIDNEY DISEASE AND ITS THERAPEUTIC MANAGEMENT IN DOGS**” submitted by **Kuljeet Singh Dhaliwal (L-2019-V-54-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc.**, in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Surgery and Radiology**) has been approved by the Student’s Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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### ABSTRACT

The present study was aimed with the objective to undertake early diagnosis and to evaluate treatment in acute kidney disease (AKD) in dogs. Thirty dogs with AKD (SCr >1.7 mg/dl and BUN >25 mg/dl) and 20 apparently healthy dogs were selected for the study. Ten dogs were in AKD IRIS grade 2, 12 in grade 3, 7 in grade 4 and one dog in grade 5. Maximum dogs were in the age group of < 4 years (n=15, 50%), followed by 4-8 years (n=12, 40%) and 8-12 years (n=3, 10%). Mean Hb, PCV, TEC levels were significantly lower while mean TLC, absolute neutrophil & lymphocyte counts and N:L were significantly ( $P \leq 0.05$ ) higher in AKD dogs than the control group. Mean serum BUN, Cr,  $P_i$ , Cr/ $P_i$ , SDMA, NGAL and Cystatin C levels were significantly higher and BUN/ Cr & Ca/ $P_i$  lower in AKD dogs. Mean protein, UP:UC, uric acid, GGT, ALKP, SDMA and NGAL in urine were significantly higher in AKD dogs. On ultrasonography, 12/19 had normal contour & echotexture of kidney, 6 had increased cortical echogenicity, 2 cases with NSAID toxicity had increased cortical echogenicity along with medullary rim sign and renomegaly in one dog. Hypostenuria (urine sp gr < 1.015) was observed in 17 dogs and isothermia (urine sp gr 1.015-1.030) in 13 dogs. Microscopic examination of urine revealed, renal epithelial cells (n=9), urothelial cells (n=12), RBC's (n=5), WBC's (n=3), granular cast (n=10), waxy cast (n=6), hyaline cast (n=8), granular + waxy cast (n=2), crystals of phosphate (n=5) and calcium oxalate (n=2). On urine culture, *E. coli* was found in 4 dogs, *Staphylococcus sp.* in 3, *Proteus sp.* in 2 and *Streptococcus sp.* in one dog. On blood smear examination, 2 dogs were positive for *Ehrlichia canis*, while on PCR analysis, 5 dogs were positive for hemoprotozoa (*Babesia canis vogeli*, n=1) and rickettsia (*Ehrlichia canis*, n= 4). On post mortem examination, splenomegaly along with renal cyst was observed in one dog, while hypertrophied right kidney and necrotic bladder wall was observed in second dog and histopathology suggested glomerulonephritis. The probable etiological factors identified in AKD dogs were: undefined etiology (n=10, 33.33%), cystitis (n= 4, 13.33%), Arthritis (n=3, 10%), *E. canis*, NSAID intoxication, pyometra, ascites (each n=2, 6.6%) and *B. canis*, Ascites+ *E. canis*, Septic wound, IMHA+ *E. canis* and Post-surgery (each n=1, 3.33%). In dogs managed with IHD along with conventional treatment, total mean blood volume processed during first session was  $155.36 \pm 42.57$  ml/kg, mean CrRR was  $28.85 \pm 5.86$  per cent and URR was  $36.8 \pm 15.9$  per cent. Out of 26 cases which were managed with conventional treatment, 20 dogs recovered during course of treatment and 6 dogs died within a week.

**Keywords:** Dogs, renal failure, renal insufficiency, acute kidney disease, acute renal failure, CBC, RFT, urine, renal biomarkers, SDMA, hemodialysis, treatment

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Signature of Major Advisor

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Signature of the student

## CONTENTS

| CHAPTER | TOPIC                   | PAGE NO. |
|---------|-------------------------|----------|
| 1       | INTRODUCTION            | 1 – 5    |
| 2       | REVIEW OF LITERATURE    | 6 – 31   |
| 3       | MATERIALS AND METHODS   | 32 – 44  |
| 4       | RESULTS AND DISCUSSION  | 45 – 87  |
| 5       | SUMMARY AND CONCLUSIONS | 88 – 93  |
|         | REFERENCES              | 94 – 111 |
|         | VITA                    |          |

## LIST OF TABLES

| Table No. | Title                                                                                                 | Page No. |
|-----------|-------------------------------------------------------------------------------------------------------|----------|
| 1         | Age wise distribution of healthy dogs (n=20)                                                          | 32       |
| 2         | Age wise distribution of dogs with acute kidney disease (n=30)                                        | 33       |
| 3         | Gender wise distribution of dogs with acute kidney disease (n=30)                                     | 33       |
| 4         | Composition of master mix for PCR assay of <i>B. gibsoni</i>                                          | 37       |
| 5         | Composition of master mix for PCR assay of <i>B. vogeli</i>                                           | 37       |
| 6         | Composition of master mix for PCR assay of <i>H. canis</i>                                            | 38       |
| 7         | Composition of master mix for PCR assay of <i>E. canis</i>                                            | 39       |
| 8         | Cycling conditions of PCR for different hemoproteoan and rickettsia                                   | 40       |
| 9         | Treatment protocol for dogs with acute kidney disease (AKD) (n=30)                                    | 43-44    |
| 10        | Age wise and IRIS stage distribution of dogs presented with AKD as per IRIS staging of AKI (n=30)     | 46       |
| 11        | Gender and IRIS stage wise grouping of dogs presented with acute kidney disease (n=30)                | 48       |
| 12        | Breed and IRIS stage wise distribution of dogs with acute kidney disease (n=30)                       | 48       |
| 13        | Clinical signs in dogs presented with acute kidney disease (n=30)                                     | 50       |
| 14        | Vital body parameters in healthy (n=20) and acute kidney disease dogs (n=30)                          | 52       |
| 15        | Vital body parameters in AKD dogs as per IRIS staging and UP:UC criteria                              | 53       |
| 16        | Haematological parameters in dogs with acute kidney disease (n=30) as per IRIS staging                | 55       |
| 17        | Haematological parameters in dogs with acute kidney disease (n=30) as per ACVIM and UP: UC staging    | 56       |
| 18        | Blood biochemical parameters in dogs with acute kidney disease (n=30) as per IRIS staging             | 57-59    |
| 19        | Blood biochemical parameters in dogs with acute kidney disease (n=30) as per ACVIM and UP: UC staging | 60-62    |

| <b>Table No.</b> | <b>Title</b>                                                                                                                         | <b>Page No.</b> |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 20               | Pearson's correlation test of serum creatinine in relation to various haematobiochemical, serum and urine biomarkers                 | 64              |
| 21               | Serum renal biomarkers in dogs with acute kidney disease (n=20) as per IRIS staging                                                  | 66              |
| 22               | Serum renal biomarkers in dogs with acute kidney disease (n=20) as per ACVIM and UP: UC staging                                      | 67              |
| 23               | Urine renal biomarkers in dogs with acute kidney disease (n=20) as per IRIS staging                                                  | 70              |
| 24               | Urine renal biomarkers in dogs with acute kidney disease (n=20) as per ACVIM and UP: UC staging                                      | 71              |
| 25               | Ultrasonographic findings in dogs with acute kidney disease (n=19)                                                                   | 72              |
| 26               | Radiographic findings in dogs affected with acute kidney disease (n=8)                                                               | 74              |
| 27               | Urinalysis in dogs affected with acute kidney disease as per IRIS staging (n=30)                                                     | 75-76           |
| 28               | Urine culture and antibiotic sensitivity test in dogs affected with AKD (n=10)                                                       | 80              |
| 29               | Hemoprotozoan and rickettsia infection positive on blood smear examination and PCR analysis in dogs with acute kidney disease (n=30) | 81              |
| 30               | Probable etiology in dogs with acute kidney disease (n=30)                                                                           | 83              |
| 31               | Intermittent haemodialysis treatment in dogs with acute kidney disease (n=4)                                                         | 84              |
| 32               | Haematological and biochemical analysis during pre-treatment and post-treatment of dogs affected with acute kidney disease (n=20)    | 85              |

## LIST OF FIGURES

| <b>Fig. No.</b> | <b>Title</b>                                                                                               | <b>Page No.</b> |
|-----------------|------------------------------------------------------------------------------------------------------------|-----------------|
| 1               | Age wise distribution of dogs affected with acute kidney disease (n=30)                                    | 47              |
| 2               | Age and IRIS stage wise distribution of dogs affected with acute kidney disease as per IRIS staging (n=30) | 47              |
| 3               | Breed wise distribution of dogs affected with acute kidney disease (n=30)                                  | 49              |
| 4               | Clinical signs in dogs affected with acute kidney disease (n=30)                                           | 51              |
| 5a              | Sensitivity of serum SDMA in ELISA                                                                         | 65              |
| 5b              | Sensitivity of serum NGAL in ELISA                                                                         | 65              |
| 5c              | Sensitivity of serum cystatin C in ELISA                                                                   | 65              |
| 5d              | Sensitivity of serum cTn-1 in ELISA                                                                        | 65              |
| 6a              | Sensitivity of urine SDMA in ELISA                                                                         | 69              |
| 6b              | Sensitivity of urine cystatin C in ELISA                                                                   | 69              |
| 6c              | Sensitivity of urine NGAL in ELISA                                                                         | 69              |
| 7               | Ultrasonographic findings in dogs with acute kidney disease (n=19)                                         | 73              |
| 8               | Urine specific gravity (Refractometer) in dogs affected with acute kidney disease (n=30)                   | 77              |
| 9               | Microscopic examination (cells) in urine in dogs affected with acute kidney disease (n=30)                 | 77              |
| 10              | Microscopic examination (casts and crystals) of urine in dogs affected with acute kidney disease (n=30)    | 78              |

## LIST OF PLATES

| Plate No. | Title                                                                          |
|-----------|--------------------------------------------------------------------------------|
| 1         | ELISA microtiter plate                                                         |
| 2         | Standard curve of ELISA                                                        |
| 3         | Flowchart of DNA isolation procedure                                           |
| 4         | Refractometer reading of urine specific gravity                                |
| 5a        | Petechial hemorrhages                                                          |
| 5b        | Hemoptysis                                                                     |
| 5c-d      | Necrosis of tip of tongue                                                      |
| 6a        | Increased cortical echogenicity of kidney                                      |
| 6b        | Medullary rim sign                                                             |
| 6c        | Renal cyst                                                                     |
| 6d        | Nephrolith                                                                     |
| 7a        | Triple phosphate crystals in urine on microscope examination (40X)             |
| 7b        | Hyaline cast in urine on microscope examination (40X)                          |
| 7c        | Bilirubin cast in urine on microscope examination (40X)                        |
| 7d        | RBC's in urine on microscope examination (40X)                                 |
| 8a        | <i>E. canis</i> on blood smear examination (100X)                              |
| 8b        | <i>B. canis</i> on blood smear examination (100X)                              |
| 8c        | <i>E. canis</i> positive on spot on kit test                                   |
| 8d        | <i>B. canis vogeli</i> (601 bp- Arrow) on PCR                                  |
| 8e        | <i>E. canis</i> (380 bp-Arrow) on PCR                                          |
| 9a        | Right hypertrophied kidney on post-mortem examination                          |
| 9b        | Cortico-medullary cyst on post-mortem examination                              |
| 9c        | Splenomegaly on post-mortem examination                                        |
| 9d        | Necrotic urinary bladder on post-mortem examination                            |
| 9e        | Glomerular swelling on microscopic examination (100X)                          |
| 9f        | Tubular and glomerular cellular infiltration on microscopic examination (100X) |
| 10a       | Dialysis tubing's and catheter                                                 |
| 10b       | Central Venous Catheter placement (pre-dialysis)                               |
| 10c       | Intermittent hemodialysis in dog                                               |
| 10d       | Working dialysis apparatus                                                     |

## LIST OF ABBREVIATIONS

|                        |                                                    |
|------------------------|----------------------------------------------------|
| %                      | : Percentage                                       |
| @                      | : at the rate                                      |
| <                      | : Less than                                        |
| >                      | : More than                                        |
| ±                      | : Plus minus                                       |
| ≤                      | : Less than or equals to                           |
| ≥                      | : More than or equals to                           |
| μL                     | : Microliter                                       |
| μg / dL                | : Microgram per decilitre                          |
| μM                     | : Micromole                                        |
| ACE-inhibitors         | : Angiotensin Converting Enzyme Inhibitors         |
| ACVIM                  | : American College Of Veterinary Internal Medicine |
| ADMA                   | : Asymmetric Dimethyl Arginine                     |
| ADQI                   | : Acute Dialysis Quality Initiative                |
| AKD                    | : Acute Kidney Disease                             |
| AKI                    | : Acute Kidney Injury                              |
| AL(OH) <sub>3</sub>    | : Aluminium hydroxide                              |
| ALB                    | : Albumin                                          |
| ALKP                   | : Alkaline phosphatase                             |
| ALP                    | : Alkaline phosphatase                             |
| ALT                    | : Alanine aminotransferase                         |
| AMP                    | : Ampicillin                                       |
| AMX                    | : Amoxicillin                                      |
| AMX-CL                 | : Amoxicillin-clavulanic acid                      |
| ANOVA                  | : Analysis of Variance                             |
| ARF                    | : Acute Renal Failure                              |
| AST                    | : Aspartate aminotransferase                       |
| ATN                    | : Acute tubular necrosis                           |
| AW I                   | : Wash buffer 1                                    |
| AW II                  | : Wash buffer 2                                    |
| B – Mode               | : Brightness modulation                            |
| <i>B. canis vogeli</i> | : <i>Babesia canis vogeli</i>                      |
| <i>B. gibsoni</i>      | : <i>Babesia gibsoni</i>                           |
| bid                    | : Twice a day                                      |
| BIL                    | : Bilirubin                                        |
| BLD                    | : Blood                                            |
| BP                     | : Blood pressure                                   |

|                 |                                          |
|-----------------|------------------------------------------|
| BUN             | : Blood urea nitrogen                    |
| BW              | : Body weight                            |
| Ca              | : Calcium                                |
| Ca/P            | : Calcium/ phosphorus                    |
| CaO             | : Calcium oxalate                        |
| CBC             | : Complete blood count                   |
| CFT-SB          | : Ceftriaxone-sulbactam                  |
| CIP             | : Ciprofloxacin                          |
| CKD             | : Chronic Kidney Disease                 |
| Cl <sup>-</sup> | : Chloride                               |
| COP             | : Colloidal osmotic pressure             |
| COX             | : Cyclo-oxygenase                        |
| Cr              | : Creatinine                             |
| CRF             | : Chronic Renal Failure                  |
| CRR             | : Creatinine reduction ratio             |
| cTn1            | : Cardiac troponin 1                     |
| CTZ             | : Chemoreceptor trigger zone             |
| CVC             | : Central venous catheter                |
| DAP             | : Diastolic arterial pressure            |
| DIC             | : Disseminated Intravascular Coagulation |
| DKA             | : Diabetic-ketoacidosis                  |
| DLC             | : Differentiate Leukocyte Count          |
| DNS             | : Dextrose normal saline                 |
| <i>E. canis</i> | : <i>Ehrlichia canis</i>                 |
| ECG             | : Electrocardiography                    |
| EHBO            | : Extra hepatic bile duct obstruction    |
| ELISA           | : Enzyme linked immunosorbent assay      |
| ENR             | : Enrofloxacin                           |
| g/dL            | : Gram per deciliter                     |
| g/L             | : Gram per litre                         |
| GEN             | : Gentamicin                             |
| GFR             | : Glomerular filtration rate             |
| GGT             | : Gamma-glutamyl transferase             |
| GI              | : Gasrto-intestinal                      |
| GIT             | : Gasrto-intestinal tract                |
| GLB             | : Globulin                               |
| GLU             | : Glucose                                |
| <i>H. canis</i> | : <i>Hepatozoan canis</i>                |

|                   |                                              |
|-------------------|----------------------------------------------|
| Hb                | : Hemoglobin                                 |
| HCT               | : Hematocrit                                 |
| hpf               | : High power field                           |
| HRP               | : Horseradish peroxidase                     |
| I/M               | : Intramuscular                              |
| I/V               | : Intravenous                                |
| Ig-G              | : Immunoglobulin G                           |
| IHD               | : Intermittent haemodialysis                 |
| IL-10             | : Interleukin-10                             |
| IMHA              | : Immune Mediated Haemolytic Anemia          |
| IRIS              | : International Renal Interest Society       |
| K <sup>+</sup>    | : Potassium                                  |
| KDIGO             | : Kidney Disease Improving Global Outcomes   |
| KET               | : Ketone                                     |
| KIM-1             | : Kidney Injury Molecule-1                   |
| L                 | : Litre                                      |
| LDH               | : Lactate dehydrogenase                      |
| LEU               | : Leucocyte                                  |
| MAP               | : Mean Arterial Pressure                     |
| MAT               | : Microscopic Agglutination Test             |
| MCHC              | : Mean Corpuscular Haemoglobin Concentration |
| MCV               | : Mean Corpuscular Volume                    |
| mEq/L             | : Milli equivalent per litre                 |
| MET               | : Metronidazole                              |
| Mmol/L            | : Micromole per litre                        |
| mg                | : Milligram                                  |
| mg/dL             | : Milligram per deciliter                    |
| mg/kg             | : Milligram per kilogram                     |
| mg/L              | : Milligram per litre                        |
| Mg <sup>+</sup>   | : Magnesium                                  |
| MgCl <sub>2</sub> | : Magnesium chloride                         |
| min               | : Minute                                     |
| mL                | : Millilitre                                 |
| mM                | : Millimole                                  |
| N                 | : Number of animals                          |
| N/2 DNS           | : Dextrose with ½ strength sodium            |
| N:L               | : Neutrophil Lymphocyte Ratio                |
| Na <sup>+</sup>   | : Sodium                                     |

|                      |                                                                |
|----------------------|----------------------------------------------------------------|
| Na <sub>2</sub> EDTA | : Di-sodium EDTA                                               |
| NAG                  | : N-acetyl-D-glucosaminidase                                   |
| NFW                  | : Nucleus free water                                           |
| ng/dL                | : Nanogram per decilitre                                       |
| ng/ml                | : Nanogram per millilitre                                      |
| NGAL                 | : Neutrophil Gelatinase Associated Lipocalin                   |
| NIT                  | : Nitrite                                                      |
| NO                   | : Nitric oxide                                                 |
| No.                  | : Number                                                       |
| NSAID's              | : Non-Steroidal Anti Inflammatory Drugs                        |
| NT proBNP            | : N-Terminal proB-type Natriuretic Peptide                     |
| °F                   | : Degree Fahrenheit                                            |
| °C                   | : Degree Celsius                                               |
| OCM                  | : Online clearance mode                                        |
| OD                   | : Optical density                                              |
| od                   | : Once a day                                                   |
| PBS                  | : Hydrostatic pressure in Bowman space fluid                   |
| PCR                  | : Polymerase Chain Reaction                                    |
| PCT                  | : Proximal Convolute Tubule                                    |
| PCV                  | : Packed Cell Volume                                           |
| PD                   | : Polydipsia                                                   |
| PGC                  | : Post glomerular capillary                                    |
| PGS                  | : Glomerular capillary hydrostatic pressure                    |
| pH                   | : Power of hydrogen                                            |
| Pi                   | : Inorganic phosphorous                                        |
| Pmol                 | : picomol                                                      |
| PO                   | : per os                                                       |
| PRO                  | : Protein                                                      |
| PU                   | : Polyuria                                                     |
| RBC                  | : Red Blood Cells                                              |
| RBP                  | : Retinol Binding Protein                                      |
| RIFLE                | : Risk, Injury, Failure, Loss of kidney function and End stage |
| RNA                  | : Ribonucleic Acid                                             |
| rpm                  | : Rotation per minute                                          |
| S/C                  | : Subcutaneous                                                 |
| SAP                  | : Systolic Arterial Pressure                                   |
| SBP                  | : Systolic Blood Pressure                                      |
| sec                  | : Second                                                       |

|               |                                        |
|---------------|----------------------------------------|
| SCr           | : Serum creatinine                     |
| SDMA          | : Symmetric Dimethyl Arginine          |
| SEM           | : Standard Error Mean                  |
| SLE           | : Systemic Lupus Erythematosus         |
| SPSS          | : Standard package for social science  |
| TAE           | : Tris-acetate EDTA                    |
| TB            | : Total Bilirubin                      |
| TCC           | : Transitional Cell Carcinoma          |
| TEC           | : Total Erythrocyte Count              |
| TGF           | : Tubulo-glomerular feedback           |
| TLC           | : Total Leukocyte Count                |
| TNF- $\alpha$ | : Tumor Necrosis Factor- $\alpha$      |
| TP            | : Total protein                        |
| tsf           | : Teaspoon full                        |
| t-test        | : Hypothesis test statistic            |
| U/L           | : Units per litre                      |
| UCr           | : Urine creatinine                     |
| $\mu\text{g}$ | : Microgram                            |
| UP            | : Urine protein                        |
| UP:UC         | : Urine protein Urine creatinine ratio |
| URO           | : Urobilinogen                         |
| URR           | : Urea Reduction Ratio                 |
| USG           | : Urine specific gravity               |
| UTI           | : Urinary tract infections             |
| VD            | : Ventro-dorsal                        |
| w.r.t         | : with respect to                      |
| WBC           | : White Blood Cells                    |
| X-ray         | : X radiation                          |

## CHAPTER I

### INTRODUCTION

Kidneys serve as the principal organ in maintenance of body homeostasis, elimination of harmful metabolic wastes, regulation of blood pressure & systemic pH, and erythropoiesis (Cortan & Kumar, 1999). Renal azotemia refers to a reduction in glomerular filtration rate (GFR) of ~75% during acute or chronic primary renal (or intrarenal) diseases (Brown et al., 2013). Acute renal failure (ARF) or acute kidney injury (AKI) or acute kidney disease (AKD) is seen when a sudden, severe insult damages the kidneys. AKD can be prerenal, renal and post renal. The main causes of AKD are toxins (Ethylene glycol, aminoglycoside antibiotics, grapes, raisins, lily, NSAIDS, hypercalcemia, hemoglobinuria), ischemia (embolic showers from disseminated intravascular coagulation or severe prolonged hypoperfusion (hypotension and hypovolemia), infections (leptospirosis and pyelonephritis), obstruction of the urinary tract (urolithiasis), etc. Prolonged diminished renal perfusion/ ischemia can cause acute tubular necrosis or intrinsic renal failure. There is an abrupt and prolonged fall in the GFR causing accumulation of nitrogenous wastes along with fluid, electrolyte and acid-base imbalance (Brown et al., 2013; Cowgill & Langston 2011; Ross 2011; Vaden et al., 1997).

Among various canine diseases, renal failure is the most common and fatal condition in canine occurring in 2-5 per cent of dogs (Lund et al., 1999) and is the third leading cause of death in dogs. Tufani et al. (2017) screened 9149 dogs screened for renal diseases, out of which 100 (1.09%) were showing syndrome of renal failure. Supriya (2019) reported annual hospital prevalence of renal diseases as 4.937 per cent and that of renal insufficiency/failure ( $\text{Cr} \geq 1.4 \text{ mg/dL}$ ) as 4.63 per cent.

Mild AKI generally goes unnoticed and generally recognized in advanced stages and is clinically characterized by anorexia, depression, oral ulceration, vomiting and/or diarrhoea, or oliguria. Chronic kidney disease (CKD) is due to loss of functional renal tissue due to a prolonged ( $\geq 2$  months) and usually progressive, process. CKD often smolders for many months or years before it becomes clinically apparent, and it is invariably irreversible and frequently progressive. Because AKI may progress to a chronic condition, any cause of AKI is also a possible cause of CKD. CKD should be distinguished from the more readily reversible acute disease

by history, physical examination and laboratory findings, although a renal biopsy may be required (Brown et al., 2013).

Acute kidney injury (AKI) has 4 stages. The first, or initiation phase, occurs during and immediately after the insult to the kidneys. During second stage there is ischemia, hypoxia and inflammation leading to cellular apoptosis, necrosis, etc. Clinical and laboratory abnormalities may not be evident during the first 2 stages. The third stage is characterized by azotemia, uremia, or both and may last for days to weeks. Oliguria (<0.5 ml urine per kg body weight per hour) or anuria may occur during this stage. The fourth stage is recovery, during which azotemia improves and renal tubules undergo repair. Marked polyuria may occur during this stage (Ross, 2011). As per International Renal Interest Society (IRIS), AKI is primarily categorized into Grade I to V on the basis of serum creatinine. In Grade I AKI, animal is non-azotemic (serum creatinine  $\leq 1.6$  mg/dL). In Grades II, III, IV and V, serum creatinine levels are 1.7–2.5 mg/dL, 2.6–5 mg/dL, 5.1–10 mg/dL and >10 mg/dL, respectively (Brown et al., 2013).

CKD is generally classified into 1 to 4 stages. Generally, no clinical signs are seen until  $\geq 75\%$  of nephron function has been impaired (Stages 3 and 4). In Stage 1, a process is damaging the kidneys but azotemia and clinical signs have not developed. In Stage 2, the disease has progressed, GFR has fallen to <25% of normal, and azotemia is present, but clinical signs are not yet seen but there may be impaired urine-concentrating ability and increased urine volume. In stage 3, GFR has declined further, azotemia is present, and clinical signs are often seen. In stage 4, there is severe azotemia with clinical signs. Usually, the earliest clinical signs in CKD are polydipsia and polyuria (late Stage 2 or early Stage 3). In Stages 1 and 2 of CKD, diagnosis is often missed or made incidentally. In Stages 3 and 4, the BUN, serum creatinine, and Pi are increased and K is generally decreased. However, hyperkalemia may be associated with oliguria and anuria. The urine specific gravity may range from 1.001–1.060 in dogs. In animals with dehydration and normal renal function, it should be >1.030. The inability to produce concentrated urine in dehydration is an early sign of CKD but concentrated urine is rarely seen when the serum creatinine is >4 mg/dL in an animal with azotemia of renal origin (Brown et al., 2013). The presence of inappropriately concentrated urine (specific gravity 1.007–1.035) with dehydration and/or azotemia suggests renal dysfunction

Differentiating between AKD and CKD and establishing the cause is important for specific therapy and predict prognosis. A history of hypotension, shock, recent exposure to known nephrotoxin with sudden uremia is a typical picture of AKD. Cylindruria, normal or increased size of kidney, often absence of anemia are typical signs, while oliguria or anuria following rehydration associated with hyperkalemia is a poor prognostic sign.

Measurement of glomerular filtration rate (GFR) is the most accurate and sensitive method for early assessment of renal dysfunction (Kerl & Cook, 2005). Generally serum creatinine and urea concentration are used for the diagnosis of kidney disease, but it is insensitive, because increase in serum creatinine is mild and often remain within the reference range (Polzin, 2011). In kidney injury, normal renal physiology alters, and the mechanisms that prevent proteinuria are compromised. Damage to glomerulus causes increased permeability of the filtration barrier, resulting in increased filtration of protein molecules (D'Amico & Bazzi, 2003). However, many clearance tests are costly and time consuming and not suitable for widespread use as a screening test (Kerl & Cook, 2005). Thus, the diagnosis of renal dysfunction at an early stage poses a challenge for practitioners.

Some markers can be used in the detection of glomerular or tubular dysfunction earlier than conventional methods. An ideal biomarker should be specific, non-invasive, sensitive to allow early detection, specific of the extent / severity of disease, be suitable for monitoring progression, informative w.r.t. the localization of the injury and the clinical outcome, inexpensive and readily available from a reference laboratory (Cobrin et al., 2013; Kovarikova, 2015). Protein markers of tubular function damage and dysfunction e.g. Retinol Binding Protein (RBP), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule 1 (KIM-1) and enzymes can be markedly increased in the urine due to reduced tubular reabsorption (Cianciolo et al., 2016).

After injury, the kidney has considerable potential for functional regeneration through hypertrophy and adaptive hyperfunction. In CKD, the most of this regenerative process has occurred before the initial diagnosis, while in AKI there is more potential for improvement of renal function, if uremic episode is taken care of. Severe AKI is a serious condition, with a survival rate of ~50%. If the cause

is known, specific therapy should be instituted. Initial management steps towards AKI involve fluid resuscitation, avoidance of nephrotoxic medications, nutrition & dietary therapy, correction of anuria or oliguria, symptomatic treatment of nausea & vomiting, and correction of varied electrolyte & metabolic disturbances (Lunn, 2011).

Disorders of sodium, chloride, potassium, calcium, and phosphorus are commonly observed in canine renal failure causing life threatening conditions. Therefore, effective therapy of a uremic crisis depends upon careful management of fluid and electrolyte balance (Langston, 2008). A polyionic fluid such as lactated Ringer's solution is satisfactory unless hyperkalemia is present, in which case normal saline is recommended. Sodium bicarbonate may be added to correct acidosis. In oliguric or anuric animals, therapy to promote increased urine volume is often recommended, if the animal is well hydrated and urine production is  $<0.5$  mL/kg/hr. Administration of excess fluid in the maintenance phase of oliguria may result in pulmonary and cerebral edema. A sequential approach generally includes an initial slight overhydration by administration of a test dosage of polyionic solution. If this fails to yield adequate urine flow, osmotic diuresis with mannitol or dextrose alternate with lactated Ringer's solution may be started. Subsequently, furosemide can be tried in increasing doses if urine production does not increase above the target of 0.5 mL/kg/hr. Finally, renal vasodilators (dopamine) or calcium channel blocker (amlodipine besylate or diltiazem) may be preferred.

Peritoneal dialysis or hemodialysis may be necessary if none of the above measures restores urine production (Brown et al., 2013). Hemodialysis is an advanced extracorporeal renal replacement therapy for uremic crisis. Dialysis removes various uremic toxins, corrects electrolytic disorders, restoration of acid-base balance, and maintains necessary functioning until the renal injury is repaired. Prognosis of dogs affected with AKI managed by hemodialysis depends on several variables like disease severity, number of organs involved, and reversibility of kidney injury, which is directly affected by severity and etiology (Segev et al., 2008).

Criteria for poor prognosis of AKI case includes severe azotemia (serum creatinine  $>10$  mg/dL), hypocalcemia, anemia, oliguria, hyperphosphatemia, worsening of azotemia with adequate fluid and supportive therapy, and associated comorbid disorders like pancreatitis or sepsis (Ross, 2011).

Earlier studies on renal failure in dogs (Dogra, 2018; Supriya, 2019) at GADVASU reported main clinical signs of vomiting, melena, polyuria, polydipsia and halitosis. Echocardiography indicated left ventricular hypertrophy. Ultrasonography revealed hyperechoic renal cortices and loss of corticomedullary differentiation. Hematology revealed anemia, high TLC and neutrophilia. Serum Cr, BUN and Pi. levels were increased. Urine analysis revealed increased UP:UC and microalbuminuria. Levels of serum SDMA, cTnl & NTproBNP and urine SDMA increased. The serum SDMA and cystatin C were positively correlated. *Babesia gibsoni*, *Hepatozoon canis*, *Babesia vogeli*, *Ehrlichia canis* were main hemoprotozoan and rickettsial etiological agents.

Though a number of studies on kidney failure have been undertaken in India and at GADVASU, Ludhiana but there is scarcity of literature on early diagnosis of kidney disease and its therapeutic management in dogs. The present study was planned in dogs with the following objectives:

1. To undertake early diagnosis of acute kidney disease in dogs.
2. To evaluate treatment with or without hemodialysis in acute kidney disease in dogs.

## **CHAPTER II**

### **REVIEW OF LITERATURE**

#### **2.1 Definition**

Acute Renal failure (ARF) is described as a sudden loss of the kidneys' ability to excrete wastes, concentrate urine, preserve electrolytes, and maintain fluid balance and may lead to higher mortality (50% and 80 %) (Han et al., 2002).

Acute renal failure (ARF) occurs when glomerular filtration declines abruptly and for an extended period of time, resulting in the accumulation of nitrogenous wastes (Kyles et al., 2005).

Acute kidney injury (AKI) is defined as the decline in the renal filtration rate followed by rise in S.Cr, uremia and oliguria. AKI affects canines in same way as it affects humans, and may be linked to one or more contributory factors (Brown et al., 2015). Hemodynamic decline (hypotension and hypovolemia), infectious (leptospirosis and pyelonephritis), nephrotoxins (nonsteroidal anti-inflammatory drugs and lily poisoning) and obstruction of the urinary tract (urolithiasis, inflammation) are the most frequently recorded AKI factors (Lee et al., 2012).

#### **2.2 Physiology of kidneys**

The kidney comprises of two major regions, the outer cortex and inner medulla which adjoins the central pelvis. The functional unit of the kidney is nephron, which comprises of the glomerulus (within the Bowman's capsule), the proximal tubule, loop of Henle, distal tubule and collecting duct. The glomerulus and Bowman's capsule are located in the renal cortex and the tubules run between the cortex and medulla. The feline kidney and canine kidney are reported to contain approximately 200,000 and 400,000 nephrons, respectively (Finch, 2014).

The normal functioning of the kidneys is to maintain homeostasis and the excretion of various metabolic waste products is dependent on various factors like adequate blood supply, production of an ultra-filtrate of plasma and the excretion of metabolic waste products. The ability to change the composition of the filtrate via reabsorption from and secretion into the tubule varies greatly (Seldin & Giebisch, 2000).

The kidneys receive approximately 25 per cent of cardiac output (>1 litre/min), but the distribution is rather not uniform. The glomerular capillaries and

peritubular capillaries that surround the proximal and distal convoluted tubules supply nearly 100 percent of the cortex (Dagher et al., 2003). The Starling forces that operate across the glomerular capillary wall are involved in the formation of an ultra-filtrate of plasma. Hydrostatic pressures in the glomerular capillary (PGC) and colloid osmotic pressure in Bowman's space are the forces that promote fluid movement out of the glomerular capillary (PBS). Colloid osmotic pressure (COP) and intrarenal pressure (primarily hydrostatic pressure in Bowman's space) are the forces that oppose fluid movement from the glomerular capillary (PBS). As a result, net ultrafiltration pressure can be expressed as  $(PGC - PBS) - (COP - PBS)$ .

The kidney's functions are diverse and thus essential for maintaining homeostasis. These functions include regulation of electrolyte and acid-base balance, water balance, arterial blood pressure, excretion of metabolic wastes, hormones and exogenous compounds (e.g., drugs), production of erythropoietin, active vitamin D synthesis and gluconeogenesis, etc. (Eaton & Pooler, 2009).

The glomerular tuft is positioned inside the cortical regions, between the arterioles, and is a site for active filtration. The resultant filtrate passes through Bowman's capsule, and its composition varies as it passes through renal tubules (Wallace, 2010).

The normal blood supply to the kidney is very heterogeneous; approximately 90% enters the cortex, which is primarily responsible for flow-dependent functions such as glomerular filtration and tubular transport. Thus, even mild perfusion change results in significant kidney functioning changes (Bankir et al., 1998).

With mild renal hypo-perfusion, compensatory mechanisms consisting of afferent vasodilation (primarily mediated by prostaglandins, NO production, myogenic TGF components of autoregulation) and efferent vasoconstriction (primarily mediated by angiotensin II) can regulate GFR temporarily (Nissenson, 1998).

### **2.3 Pathogenesis of acute renal failure (ARF)**

ARF in dogs can be due to prerenal, intrinsic renal and post renal disorder. ARF can be caused by any factor which leads to the reduced renal perfusion, such as shock, decreased cardiac output, and renal vessel thrombosis or occlusion. Renal

ischemia, or reduced renal perfusion for an extended period of time, may result in acute tubular necrosis or intrinsic renal failure in dogs (English, 1974).

Acute renal failure (ARF) occurs when glomerular filtration declines abruptly and for a considerable time period resulting in the accumulation of nitrogenous wastes in the body. This condition is often accompanied by fluid, electrolyte and acid-base disturbances (Brezis et al., 1991).

Induction, maintenance and regeneration are the three stages of acute renal failure. Oliguria has been linked to the repair process, while enhanced renal function and increased urine production have been linked to the recovery phase. A poor prognosis is associated with failure to convert from anuria or oliguria to a non-oliguric condition and thus may require immediate dialytic therapy (King et al., 1994).

Renal failure is a condition in which azotemia and the inability to produce hypersthenuric urine coexist. Acute renal injury and chronic kidney disease require different diagnostic approaches and thus the prognosis varies. Acute renal failure can develop in a matter of hours to days. Enlarged and swollen kidneys, hemconcentration, ideal body condition, active urine sedimentation, relatively severe hyperkalemia and metabolic acidosis, and relatively severe clinical signs for the degree of azotemia are some of the classic symptoms and pathological findings often associated with ARF (Grauer, 2005).

Decreased renal perfusion or hypo-perfusion injury followed by higher tubular pressure leading to cytosolic injury, thus acute tubular necrosis (ATN) are some of the major pathophysiologic mechanisms that contribute to ARF in dogs (Eriksen & Ingebretsen, 2006).

Renal failure can be caused by a variety of factors including existing renal diseases, ischemia, toxins, and other events that reduce renal function, as well as nephritis, urinary tract obstruction, and lower urinary tract infections (Katoch et al., 2017).

Renal damage may be caused by hypo-perfusion, renal ischemia, nephrosis and is also caused by a variety of systemic manifestations such as cardiovascular, hepatic, and diabetes mellitus (Tufani et al., 2015).

When the GFR decreases due to reduced renal blood flow or increased renal vascular resistance, usually prerenal disease occurs. Intrinsic renal failure is caused by

damage to the renal parenchyma caused by various ischemic, glomerular or tubular disorders. Post renal causes generally includes urinary tract obstruction or rupture, urolithiasis, neoplasia, trauma, inflammation, fibrosis, blood or mucus plugs (Stokes & Bartges, 2006).

Many drugs commonly used in critical care (NSAIDs, beta-lactams, sulphonamides, loop diuretics, thiazides, quinolones, cimetidine, allopurinol, proton pump inhibitors) are linked to acute interstitial nephritis and account for 3-15 per cent of all drug-induced ARF cases (Rossert, 2001). In about 19-25 per cent cases of acute renal failure in critically ill patients, nephrotoxic drugs may be a contributing factor in the course of AKI (Mehta et al., 2004).

Pyometra is one of the most common causes of renal disease in canines. Endotoxins produced by bacteria in the uterine tissues, affects kidneys via general circulation may result in vasculitis causing inflammation or post infection immune mediated glomerulonephritis, tubular necrosis and atrophy, ultimately causing renal insufficiency (Heiene et al., 2007).

### **2.3.1 Phases of ARF**

ARF is divided into three phases: (1) initiation (2) maintenance and (3) recovery. Therapeutic measures that reduce the renal insult during the initiation phase can help to prevent the development of ARF (Grauer, 2005). The initiation being characterised by decreased renal flow, reduced tissue oxygenation and urine output less than 0.5ml/kg/hr.

The maintenance phase is characterized by various tubular lesions and established nephron dysfunction. Although potentially life-saving, therapeutic intervention during the maintenance phase usually has little effect on existing renal lesions or dysfunction. The recovery phase is when the renal lesions heal and the function of the kidneys improves or regains (Grauer, 1996). The recuperation stage generally keeps going for days beginning with post-acute tubular necrosis diuresis.

### **2.3.2 Risk factors for acute renal damage**

The most common and important risk factors for the development of ARF are dehydration, reduced PCV and volume depletion. Pre-existing renal disease and advanced age can also contribute to the onset of the condition or can exaggerate the condition (Vaden et al., 1997).

Many other risk factors have also been associated with the development of ARF and includes pre-existing chronic disease, hypo-volemia with decreased renal blood flow, DIC, cytotoxic effects of certain vasoactive substances and concurrent systemic infection (Tran et al., 1993).

### **2.3.3 Outcome of ARF**

Dogs with gastrointestinal symptoms (vomiting or diarrhoea) or abnormal electrolyte metabolism (high phosphorus, potassium and low chloride levels) had a consistently poor prognosis because such dogs were found to be more likely to succumb to AKI (Lee et al., 2011).

## **2.4 Pathogenesis of Chronic renal failure**

Chronic kidney disease (CKD) has been defined and classified in various ways over time, but current international guidelines define it as decreased kidney function manifested by the glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 sq.m (Webster et al., 2017).

According to Bhojne (2016), renal damage that has been present for at least three months, with or without a decreased GFR or a reduction in GFR of more than 50% from normal, and that has been present for at least three months is CKD in dogs. Common signs of CKD are progressive weight loss, melena, halitosis and normochromic normocytic non-regenerative anemia.

The issues of the macrovascular compartment (hypertension, coagulopathies and ongoing hypo-perfusion), microvascular compartment (glomerular hypertension) and interstitial compartment (pyelonephritis, tumours, obstructive uropathies, nephritis) are associated with end stage renal or persistent kidney illness (Brown et al., 2007).

Brito-ashurst et al. (2009) noted that proteinuria and arterial hypertension are related to the poor prognosis and thus irreversible damage to kidneys was evident.

Tufani et al. (2015) studied ten dogs with end-stage kidney disease which were having severe azotemia, hydronephrosis, renomegaly, nephrolithiasis, nephrocalcinosis and renal cyst.

Polyuria, polydipsia and anorexia were the most common clinical symptoms in the stage I and stage II groups. The most common symptoms observed in stage III

dogs were anorexia, vomiting, melena, emaciation, pale mucus membrane and halitosis. Depression, emaciation, stomatitis, dehydration, vomiting, melena, and nervous signs were among the major clinical signs observed in stage IV dogs with end-stage renal failure (Bhadesiya et al., 2017). The disease tends to progress in dogs with IRIS CKD Stages 3 and 4. The majority of dogs with this severe CKD will die or be euthanized as a result of their illness (Roudebush et al., 2010). Dogs with IRIS CKD Stages 3 and 4 normally live for few months to a year or two, depending on the severity of their kidney disease, but with early diagnosis and thus successful treatment, survival periods may be significantly increased (Polzin, 2013).

The presence or absence of kidney damage, urine output, hypertension and changes in GFR are all key factors determining the prognosis as well as course of the disease in dogs (Barnett & Cummings, 2018).

## **2.5 Prevalence of renal failure in dogs**

### **2.5.1 Worldwide prevalence**

In a prospective survey conducted by Kyles et al. (2005) on 99 dogs, 97 dogs had one or more of morphologic evidence of acute renal lesions (45%), usual serum creatinine concentration within 1 month prior to ARF diagnosis (43%), reports of serum creatinine concentrations returning to within the reference range (19%) or documented recent nephrotoxic exposure or ischemic event (72%).

### **2.5.2 Indian scenario**

Over a two-year duration Sosnar et al. (2003) observed a cumulative prevalence of renal failure as 11.9 per cent.

Goel (2017) observed 1.47 per cent prevalence of renal insufficiency in canines out of which 30.23 per cent cases were of acute injury and 69.77 per cent were chronic renal sickness.

Katoch et al. (2017) in a study of 2277 canines, observed 108 cases of acute renal failure suggesting 4.74 per cent prevalence.

During the one-year study period of 29,159 dogs by Thade et al. (2019), 271 dogs (0.93 %) were found to be azotemic.

Supriya (2019) observed 9 ARF cases out of 90 cases of renal failure.

### **2.5.3 Age wise prevalence**

Acute renal failure is the most common disease in dogs, accounting for higher prevalence in 4 to 8 years of age (Tufani et al., 2017).

Nabi et al. (2014) observed higher prevalence of AKI in dogs over ten years of age. Thade et al. (2019) observed highest prevalence of 44.28 percent has been recorded in age range of 5-10 years followed by dogs older than 10 years (40.96 %) and lowest prevalence in dogs aged 5 years (14.76 %). Supriya (2019) observed that maximum dogs suffering from renal insufficiency were in the age group of 4-8 years.

### **2.5.4 Breed wise prevalence**

Mixed-breed dogs (27.67%), Labrador retriever (25.09%), Pomeranian (13.28%), German shepherd (9.59%), Spitz (9.59%), Golden retriever (2.95%), Dalmatian (2.21%), Rottweiler (1.85%), Pug (1.85%), Doberman pinscher (1.48%) and Saint Bernard (1.48%) were the orderly sequence of breeds affected by ARF (Pelander et al., 2015). Labrador retriever were most affected (36.66%) followed by German shepherd (21%) and so on (Supriya, 2019).

### **2.5.5 Gender wise prevalence**

Males were found to have a higher prevalence of renal diseases (60.89%) than females (39.11%) (Neill et al., 2013).

In a study of 289 dogs, Mukherjee et al. (2014) observed that females (58.60%) had a higher prevalence of renal disease than males (41.40%).

Renal failure was observed more in male dogs (76.66%) than in female dogs (23.33%) by Supriya (2019).

### **2.5.6 Diet**

No causal association was observed between the age of onset of renal failure and diet (Stanley et al., 1984). However, Kumar et al. (2011) reported that excessive protein consumption can result in nephron degeneration and eventually, renal insufficiency in dogs.

## **2.6 Renal failure classification**

The Acute Dialysis Quality Initiative (ADQI) proposed various concepts of classification of acute renal failure in dogs. The division includes concepts of GFR and urine output measurement to classify various groups according to severity i.e., risk, injury and failure (Lameire & Hoste, 2004).

ADQI working group created a multi-level classification scheme. The measure is known as RIFLE, which stands for Risk of Renal Dysfunction, Kidney Injury, Failure of Renal Function, Loss of Renal Function, and End Stage Renal Disease. This scheme graded patient's renal function into three severity categories at the same time (Lee et al., 2012).

There are three levels of severity: those with mildly impaired renal function (Risk class), those with moderately impaired renal function (Injury class) and those with extreme renal impairment falls in Failure class (Biesen et al., 2006).

### **2.6.1 RIFLE grading of acute kidney injury (AKI)**

The RIFLE (Risk, Injury, Failure, Loss and End-Stage Renal Failure) and Acute Dialysis Quality Initiative's proposed basic classification scheme for ARF as follows (Bellomo et al., 2004):

- (i) Risk:  $\geq 1.5$  times increase Cr from baseline or 50% to 25% reduction in GFR.
- (ii) Injury:  $\geq 2.0$ -times increase Cr from baseline or 75% to 50% reduction in GFR.
- (iii) Failure:  $\geq 3.0$ -times increase Cr from baseline or reduced GFR  $\geq 75\%$ , or an absolute serum creatinine  $\geq 354 \mu\text{mol/l}$  or with an acute rise of at least  $44.2 \mu\text{mol/l}$ .

### **2.6.2 AKIN grading of acute kidney injury (AKI)**

Acute Kidney Injury Network (AKIN) suggested an updated version of the RIFLE requirements in 2007. A smaller improvement in SCr within 48 hours of over  $0.3 \text{ mg/dL}$  ( $26.2 \text{ mol/L}$ ) was suggested as the threshold for AKI in AKIN stage-1 (analogous to RIFLE-Risk). Furthermore, patients on renal replacement therapy were reclassified as AKIN stage-3 (RIFLE- Failure). Finally, the types of failure and end-stage kidney disease were removed from the AKIN classification (Soliman, 2012).

### **2.6.3 IRIS grading of acute kidney injury (AKI)**

IRIS scaling grades the dogs into various stages to determine the prognostic factors in kidney patients. Based on fasting blood creatinine determination and parameter such as urine flow rate measurement, there are five classification schemes for acute kidney injury (Segev et al., 2016)

- (i) Grade 1: Defines non-azotemic patients (serum creatinine  $<1.6 \text{ mg/dl}$ ), Progressive increase in creatinine level by  $\geq 26.4 \mu\text{mol/l}$  ( $\geq 0.3 \text{ mg/dl}$ ) over 48

hour interval and oliguria ( $>1$  ml/kg/hr) or decrease in creatinine values over 48 hours.

- (ii) Grade 2: Mild azotemia (serum creatinine 1.7 to 2.5 mg/dl), the progressive increase in creatinine  $\geq 26.4$   $\mu\text{mol/l}$  ( $\geq 0.3$  mg/dl) over 48 hour interval associated with pre-existing insufficiency
- (iii) Grade 3: Moderate to severe azotemia (serum creatinine 2.6–5 mg/dl or 141 – 220  $\mu\text{mol}$ ).
- (iv) Grade 4: Moderate to severe azotemia (serum creatinine 5.1–10 mg/dl or 440 - 880  $\mu\text{mol}$ ).
- (v) Grade 5: Severe azotemia (serum creatinine  $>10$  mg/dl or  $>880$   $\mu\text{mol}$ ) with severe kidney dysfunction.

#### **2.6.4 KDIGO grading of acute kidney injury (AKI)**

KDIGO (Kidney Disease Improving Global Outcomes) emphasizes on AKI risk, preventing and treating AKI and dialytic therapies for AKI care (Khwaja, 2012). The KDIGO basis for ARF is:

- (i) Stage 1: 1.5–1.9 ( $\geq 0.3$  mg/dl) fold rise in serum creatinine from baseline or oliguria (less than 0.5 ml/kg/hr).
- (ii) Stage 2: 2.0–2.9 fold rise in serum creatinine from baseline and urine output less than 0.5 ml/kg/hr over interval of  $\geq 12$  hours.
- (iii) Stage 3: 3 ( $\geq 4.0$  mg/dl) fold rise in serum creatinine from baseline or urine output less than 0.3 ml/kg/hr over interval of  $\geq 24$  hours or anuria for  $\geq 12$  hours.

#### **2.6.5 IRIS grading of acute kidney injury (AKI) according to blood pressure**

The IRIS classification on the basis of systolic blood pressure (SBP) is as follows: normotensive (140 mm Hg) at lower risk, prehypertensive (140 – 159 mm Hg) at medium risk, hypertension (160 – 179 mm Hg) at moderate risk and severely hypertensive dogs ( $>180$  mm Hg) at higher risk.

There was a positive correlation between the severity of systemic hypertension and unfavourable renal alterations. When the dogs were divided into groups based on the severity of their systemic hypertension, more hypertensive dogs had significantly lower GFR values, higher UP:UC scores and more prominent renal lesions scores than the less hypertensive dogs. The data provided circumstantial evidences for the

negative effects of systemic hypertension on the kidneys of dogs with impaired renal function (Finco, 2004).

## **2.7 Etiology of renal failure**

ARF is an abrupt loss of renal function. It is characterised by a rapid onset of renal insufficiency, decrease in GFR and a decrease in the plasma flow. The clinical and biochemical consequences of excretory failure result in toxic waste products build-up in the bloodstream. Pre-existing renal illness, ischemia, toxins and other factors reducing renal perfusion are all potential causes of ARF (Schrier et al., 2004).

Failure to detect ARF quickly and undertake aggressive therapy may end up in irreparable renal function damage and ultimately patient's death. However, under intensive treatment and identifiable etiology, ARF may be reversible. Infectious disorders (leptospirosis, borreliosis and babesiosis), nephrotoxics (aminoglycosides, vitamin D and nonsteroidal anti-inflammatory medications) and plant material (lilies and raisins/grapes) have all been cited as causes of ARF in dogs and cats (Bhojne, 2016).

ARF can also be caused by immune-mediated illness like SLE, amyloidosis, pyelonephritis, hypercalcaemia, urinary tract blockage and infectious causes (e.g., leptospirosis) (Segev et al., 2016).

Leptospirosis, caused by serovars of *Leptospira interrogans*, is a common recognised cause of acute renal failure (ARF) in dogs (Beckel et al., 2005).

Pyelonephritis was diagnosed in 2 per cent of dogs diagnosed with ARF (Kyles et al., 2005).

AKI can be caused by specific disorders like pancreatitis because inflammatory cytokines can cause decreased renal perfusion and apoptosis of renal tubular cells (Cuthbertson & Christophi, 2006).

Frequently encountered causes of AKI were leptospirosis (74.4%, 29/39), intoxication (15.4%, 6/39; grape [4/6], ethylene glycol [1/6] and gentamicin [1/6]), obscure etiology (5.1%, 2/39), leishmaniosis and bilateral obstructive urethrolith (Cambournac et al., 2019).

Kumar et al. (2011) reported that End stage renal disorder, nephritis, urolithiasis, and hydronephrosis were identified as particular etiologies in renal disorders of dogs.

The most widely used medications that cause acute tubular necrosis are aminoglycosides, vancomycin and amphotericin B (Shriner & Wong-beringer, 2015).

The use of nephrotoxic drugs may play a role in 19-25% of cases of acute renal failure in critically ill patients (Tufani et al., 2015).

In a retrospective study, infectious and non-infectious causes of ARF were revealed. The prevalence of infectious (29.4%) etiology in ARF which included leptospirosis (43.6%), pyelonephritis (5.7%), pyometra (37.4%) and sepsis (2.5%) whereas prevalence of non-infectious (60.4%) etiology in dogs included nephrotoxic (30.3%), obstructive (36.5%) (Legatti et al., 2018).

### **2.7.1 Infectious Diseases**

#### **2.7.1.1 Leptospiral infection**

Dogs can be affected after being exposed to contaminated urine shed by wild animals, primarily rats, which serve as a maintenance host for numerous *Leptospira* serovars. The bacterium invades mucous membranes of host reaching the liver and kidneys. The most common clinical signs reported in affected dogs were icterus and haemorrhagic diathesis primarily, proceeding to renal ailment i.e., interstitial nephritis (Hutton et al., 2008).

*Leptospira* are known to cause hypokalemia, nonoliguric renal dysfunction in people because of diminished sodium reabsorption and the equivalent might happen in the canines. Urinalysis from canines with leptospirosis might reveal isosthenuria and in uncommon cases, hyposthenuria. Significant loss of protein and glucose in urine, bilirubinuria, haematuria, pyuria and cylindriuria may be present (Raila et al., 2010).

An increase in serum ALT, AST, ALP and total bilirubin levels generally indicate hepatic dysfunction, which is mostly associated with azotemic changes in blood. MAT (microscopic agglutination test) and polymerase chain reaction (PCR) are confirmatory tests (Kellum et al., 2012).

Out of total 124 sera samples from dogs of various breeds, including vaccinated, unvaccinated-semi owned and stray dogs, MAT was performed. Out of 42 vaccinated dogs 24 (57%) were positive for one or more serovars (Senthil et al., 2013).

### **2.7.1.2 Hemoprotozoan infection**

Stokes and Forrester (2004) reported that proteinuria, renal tubular casts and epithelial cells in urine sediments are common in both complex and mild babesiosis, but may not always indicate or depict renal failure.

The mechanical and toxic effects of haemoglobin on renal tubules (i.e. haemoglobinuric nephrosis) may result in the development of ARF in babesiosis (Áthé et al., 2007).

In an 11-year-old male Golden retriever diagnosed with anaplasmosis, clinical signs observed were hyperthermia, lethargy, inappetence, ataxia and oliguria. Neutrophilia, thrombocytopenia, hypercholesterolemia, elevated ALKP along with ALT, AST, GGT, BUN and SCr were common hemato-biochemical findings (Melter et al., 2007).

Bai et al. (2019) reported higher mean levels of BUN and SCr in babesiosis dogs as compared to control dogs.

### **2.7.1.3 Pyometra**

Obel et al. (1964) on necropsy of kidney of dogs affected with pyometra and observed tubulo-interstitial and glomerular lesions which were thought to be caused by immune-mediated glomerulonephritis.

Schepper et al. (1989) observed renal failure signs in dogs affected by pyometra which was characterised by increased urine excretion of protein, glucose, GGT, ALP, amylase, lipase and uroepithelial casts with high serum BUN and Cr.

Umadevi (2016) reported clinical symptoms of pyometra such as presence of unhealthy vaginal discharge (8/8), anorexia (8/8), vomiting (5/8), polyuria and polydipsia (4/8) and a normal to slightly elevated body temperature, abnormal accumulation of fluid material with varied echogenicity in the uterine horns.

### **2.7.1.4 Mycosis and mycotoxins**

Jin and Lin (2005) isolated *Candida albicans* in fungal UTI's in dogs. Lower urinary tract illnesses, diabetes mellitus, neoplasia and renal failure were the most common previous disorders seen in these affected animals. The most common clinical signs seen in the affected animals were dysuria, haematuria, and increased frequency of micturition, anorexia, dehydration, depression and pyrexia.

Ahn et al. (2007) observed nephrotoxic effects of aflatoxin (ochratoxin) in Beagle dogs with varied clinical signs including vomition, diarrhoea, polydipsia and polyuria. Hematobiochemical changes included increased levels of BUN, creatinine. Urinalysis revealed glycosuria, proteinuria and increased levels of granular cast. Biopsy revealed acute tubular necrosis in both the kidneys.

Boermans and Leung (2007) observed nephrotoxic effects of aflatoxin (ochratoxin) in Beagle dogs with varied clinical signs including vomition, diarrhoea, polydipsia and polyuria. Hematobiochemical changes included increased levels of BUN, creatinine. Urinalysis revealed glycosuria, proteinuria and increased levels of granular cast. Biopsy revealed acute tubular necrosis in both the kidneys.

Chronic administration of antibiotic and glucocorticoids, blood dyscrasias, type II diabetes, acidic urine pH, indwelling urinary catheters and certain malignant tumours pose emerging risk factors for fungal urinary tract infections (Pérez & Alessi, 2018).

## **2.7.2 Non Infectious Diseases**

### **2.7.2.1 Toxicity and poisoning**

In the early course of nephrotoxicosis, ARF occurs possibly due to reduced GFR, mediated by renin angiotensin activation (Lindner et al., 1982).

Drug toxicities is observed in about 19-24 percent of AKI (tubular necrosis) dogs with commonly administered drugs such as vancomycin, aminoglycosides, etc. (Mehta et al., 2004).

Eubig et al. (2005) between 1992 and 2002 reported 43 incidences of ARF in dogs in North America following grape or raisin intoxication and concluded that ARF can develop in dogs after consuming grapes or raisins, and that recovery of renal functioning is possible, but not all dogs recovered sufficiently to survive. Though ARF does not develop in all dogs who consume grapes or raisins, preventative measures such as generating emesis, administering activated charcoal shortly after ingestion, and commencing fluid diuresis should be considered immediately.

After contrast delivery, there is a risk of AKI. Diabetes, heart failure, volume depletion, nephrotoxic medications, and haemodynamic instability are additional risk factors in patients with contrast administration (Kane et al., 2008).

Medicines widely used in critical care (NSAIDs, beta-lactams, sulphonamides, loop diuretics, thiazides, quinolones, cimetidine, allopurinol and proton pump inhibitors, etc.), are linked to acute interstitial nephritis accounting for 3-15 per cent drug-induced ARF (Stanescu et al., 2011).

Surdyk et al. (2011) determined effects of COX inhibitors with NSAID on renal functioning. Papillary necrosis along with reduced renal prostaglandin was a causative factor in altered renal hemodynamics. Hunter et al. (2011) observed ibuprofen (81%) as most toxic followed by naproxen (11%).

Rusenov et al. (2014) reported a case of ARF associated with ethylene glycol poisoning in 11-month-old mixed breed female dog.

Aminoglycosides, vancomycin and amphotericin B may lead to acute tubular necrosis. Toxicity is caused by either accumulation or reduced elimination of drugs (Awdishu & Mehta, 2017).

Bruce & Fine (2009) observed increased levels of creatinine in groups with contrast induced nephrotoxicity.

Gleeson & Bulugahapitiya (2004) concluded that contrast-prompted nephropathy has turned into a huge wellspring of emergency clinical morbidity and mortality with the steadily expanding utilization of iodinated contrast media in imaging and interventional strategies like angiography. It is the third most normal reason for medical induced AKI.

#### **2.7.2.2 Urolithiasis**

Urolithiasis is a frequent ailment that causes lower urinary tract disease in dogs and cats. The production of bladder stones is linked to the precipitation and crystallisation of a varied range of minerals like magnesium ammonium phosphate hexahydrate, calcium oxalate, urates, etc. Over the last 15 years, urolithiasis has emerged as a significant cause of acute and chronic renal illness in cats and dogs (Brložnik & Zdovc, 2012).

Calcium oxalate is the most prevalent component of upper urinary tract stones in dogs and cats with more than 90 per cent of analysed nephrolith and urethrolith being CaO (Ross, 2011).

Because of a variety of anatomic factors, urethral blockage by uroliths is more common in male dogs than in female dogs because of a smaller relative urethral

diameter and longer urethral length, the curving path of the urethra around the ischium, and the existence of the os-penis, which limits urethral diameter expansion. Uroliths are most commonly found lodged at the ischial arch or immediately proximal to the os-penis in male dogs (Bradea et al., 2013).

#### **2.7.2.3 Neoplasms of Urinary System**

Transitional cell carcinoma (TCC) of the urinary bladder is one of the most prevalent neoplasms of the canine urinary system, accounting for 1.5-2.0 per cent of all canine malignancies. TCC is typically an aggressive and ultimately lethal cancer, with death due to post-renal blockage occurring within 3-12 months of diagnosis (Kubiak et al., 2013).

In a case of urinary bladder fibroma in a 4-year-old female Great Dane, physical examination revealed, thickened urinary bladder; pollakiuria, murky and foul-smelling urine and haematuria. The case was surgically managed, and the recovery was unremarkable (Sarma & Kalita, 2019).

#### **2.7.2.4 Traumatic injuries of UTI tract**

The most prevalent inflammatory disorders affecting external urinary organs in male dogs include posthitis, balanitis and balanoposthitis, which can either be due to direct trauma or varied infectious pathogens. Catarrhal exudation and leucocyte infiltration into the deteriorated epithelium are hallmarks of inflammatory diseases (Stokes & Bartges, 2006).

Sarma and Kalita (2019) reported two cases of phalitis associated with urolithiasis in nondescript dogs aged between 3 and 5 years.

#### **2.7.2.5 Diabetes ketoacidosis**

Causmaecker et al. (2009) identified 54 cases of diabetic ketoacidosis (DKA) based on higher levels of serum BUN and Cr, out of which 13 per cent were diagnosed with ARF of undefined etiology.

### **2.8 Clinical signs**

Azotemia is described as an increase in either serum creatinine or blood urea nitrogen (BUN) levels or both. Azotemia, renal insults, renal illness and renal failure are frequently characterised as prerenal, renal or post renal. However, uremia literally denotes the presence of urea like contents in the blood but the term is most commonly

used to refer the clinical indications that arise when azotemia progresses (Brown et al., 2007). The clinical symptoms often include a loss of appetite, vomiting, lethargic, melena and weight loss. If uremia worsens, the individuals are more prone to develop uremic gastritis, oral ulceration and platelet dysfunction or impaired platelet aggregation. Other less frequent symptoms of severe uremia include uremic pneumonitis, osteodystrophy and signs of encephalopathy (Lunn, 2011).

Rubin (1997) reported that the history and clinical signs of renal failure vary based on the nature, severity, length, degree of progression of the underlying disease, presence of co-existing but unrelated disease. Sometimes signs could be vague and non-localising such as depression, malaise, anorexia, and weight loss, dehydration, dry mucous membranes, decreased skin turgor, sunken eyeballs, etc.

Sosnar et al. (2003) stated that with upto 67 per cent loss of renal function, animal remains clinically asymptomatic and that with 67-75 per cent loss of renal function there may be polyuria and polydipsia and that loss of 75-95 per cent of renal function would be manifested by vomiting, diarrhoea, apathy and when less than 10 per cent of renal function is left, these signs may be accompanied along with encephalopathy.

Grauer (2005) claimed that acute renal failure in canines was accompanied with inflamed kidneys, hemoconcentration, metabolic acidosis, increased urinary sedimentation, marked hyperkalemia and renal pathological alterations.

Pugliese et al. (2005) stated that severe polydipsia and polyuria occurs with a loss of 67-75 per cent of the filtration rate, when renal failure further progress (75-90%), the accumulation of blood nitrogen catabolic products determined the occurrence of systemic signs such as anorexia, weight loss and specific signs such as vomiting and diarrhoea. When the residual renal function was found to be less than 10 percent, uremia was present along with neurological manifestations (uremic encephalopathy) indicating the terminal stage of illness.

Other signs of deteriorating renal function include polyuria /polydipsia, dehydration, electrolyte imbalances, acidosis, anaemia, systemic hypertension and renal secondary hyperparathyroidism (Polzin, 2011).

Klosterman et al. (2011) reported that a history of prolonged anorexia, vomiting, diarrhoea, weight loss, or a combination of these factors, polyuria /

polydipsia (PU/PD) and nocturia were more frequent in CRF. For whatever magnitude of azotemia, clinical symptoms were more severe in animals with ARF than in CRF. Emaciation, poor coat quality and mucous membrane pallor were more prevalent in CRF than in ARF.

Dilipkumar (2018) observed inappetance, vomiting along with abdominal discomfort in 5 dogs with AKD.

## **2.9 Diagnosis**

### **2.9.1 Haematology**

King et al. (1992) reported that dogs with azotemia had lower Hb and PCV levels.

Grauer (1996) reported that ARF dogs were found to have thrombocytopenia, regenerative anaemia and an inflammatory or stress leucogram.

Adin and Cowgill (2000) concluded that *Leptospira interrogans* in dogs often induced a hepatorenal syndrome defined by an acute onset of haemorrhagic diathesis, sub-acute icterus, or sub-acute uraemia followed by oliguria or anuria. Out of 37 dogs affected by leptospirosis, 17 of had neutrophilic leucocytosis.

Erythropoietin is produced by the peritubular interstitial cells of the inner cortex and outer medullary cells of the kidney. As the kidney disease progresses, there are less erythropoietin-producing cells within the kidneys, hence anemia is evident (Polzin et al., 2000).

Dorgalaleh et al. (2013) revealed RBC, Hb, HCT and MCHC levels were significantly lower in AKI affected patients along with anaemia and thrombocytopenia.

Supriya (2019) observed decreased Hb, PCV and TEC in all stages of renal failure whereas mean TLC, N: L ratio was significantly higher in renal group as compared to control group.

Mean values of TEC and Hb were lower than the normal reference range whereas, mean value of WBCs count was higher than the control group in dogs affected with AKI (Elgazzar et al., 2021).

### **2.9.2 Serum biochemistry**

Bonventre and Weinberg (2003) concluded that plasma creatinine concentration being an indirect marker of GFR is used for staging both canine and feline renal dysfunctions.

Heiene et al. (2007) concluded that serum BUN and creatinine analysis is to be used along with routine urinalysis as initial measure to evaluate renal insufficiency in canines.

GGT is eliminated in small amounts in the urine ordinarily, and tubular dysfunction dramatically increases their excretion (Braun & Lefebvre, 2008).

Ross (2011) stated that  $\text{Na}^+$  retention is seen in animals with oliguric ARF, but urine  $\text{Na}^+$  loss is higher in polyuric ARF. In renal failure, calcium levels are normally within range or slight lower, whereas phosphorus levels are generally on higher side as compared to CKD.

Cianciolo et al. (2016) reported ARF in an English setter dog caused by ethylene glycol poisoning resulted in azotemia (BUN= 126 mg/dl and creatinine = 12mg/dl) and marked hyperkalemic changes.

In the cases of renal failure caused by Babesiosis, azotemic dogs had significantly lower amounts of total proteins, albumin, beta and gamma globulins, but significantly higher quantities of alpha-2 globulin. As a result of the liver compensating for protein loss, azotemic dogs have comparatively higher serum cholesterol concentrations (Nieto, 2016).

Clinical findings in dogs with AKI included increased BUN, Cr, hyperphosphatemia, hyper-or hypokalemia and metabolic acidosis (Gwaltney-Brant, 2018).

Devipriya et al. (2018) observed significant rise in BUN, Cr, P and TP whereas albumin and calcium showed non significant difference as compared to the control group.

Goel et al. (2018) observed that all renal affected dogs had azotemia, increased alkaline phosphatase, GGT, hyponatremia, hyperphosphatemia, mild hyperkalemia and hypocalcaemia.

In dogs infected with *Ehrlichia sp.*, varied renal illness manifested by IMHA, proteinuria, neutrophilia, aberrant lymphocytosis and elevated liver enzyme activity including AST, ALT and GGT was observed by Quorollo et al. (2019).

Supriya (2019) reported higher values of mean fibrinogen in all renal affected dogs as compared to the control group and found positive correlation between serum SDMA and creatinine levels.

### 2.9.3 Urinalysis

Urinalysis is an important diagnostic tool for the urinary and renal diseases. It includes physical parameters (color, turbidity, volume, transparency), biochemical parameters (pH, blood, glucose, ketones, bilirubin, urobilinogen and protein) and microscopic assessment (RBC, WBC, epithelial cells, crystals, casts, organisms) (Harley & Langston, 2012).

Camacho et al. (2004) claimed that all dogs had azotemia of primary renal etiology if their urine specific gravity was less than 1.025.

Lulich and Osborne (2004) reported common bacteria from dog and cat urinary specimens (105 organisms per millilitre of urine) as *Escherichia coli* (33%), *Proteus* spp. (28.6%), *Staphylococcus aureus* (17 %), and *Streptococcus* spp. (7.8 %), with lesser numbers of *Enterobacter* spp. (5.3%), *Klebsiella* spp. (4.4%), *Pseudomonas aeruginosa* (2.4%) and *Staphylococcus epidermidis* (1.5%).

For renal interstitial or parenchymal disease to cause proteinuria, inflammation must be present, as in pyelonephritis, leptospirosis, renal neoplasia, and nephrolith. However, functional renal proteinuria (also known as physiologic proteinuria) is characterised by temporary, mild proteinuria that can be produced by heat, stress, seizure, venous congestion, fever, and intense exercise (Grauer, 2005).

Ross et al. (2006) stated that because the kidneys had a great reserve capacity, specific gravity could not be used as an early predictor of renal impairment. Impairment of the kidney's ability to concentrate or dilute urine will not be identified until at least two-thirds of the total population of nephrons has been significantly damaged.

More than three leukocytes per high power field in the sediment (by cystocentesis) indicate an inflammatory urinary tract condition. Granular casts in urine sediment have been recorded in 11-33 per cent of dogs suffering from acute renal damage (Cowgill & Langston, 2008).

Urine specific gravity and osmolality measurements are used to estimate the kidney's ability to concentrate urine. The ability of the chronically ill kidney to concentrate urine is dependent on appropriate antidiuretic hormone secretion and the availability of sufficient nephrons (Akmaliyah, 2013).

Hoareau et al. (2014) reported that ARF animals exhibited higher active urine sedimentation as compared to CRF animals. Nonetheless; the absence of active

sediments does not rule out diagnosis of ARF. The presence of a considerable number of urine casts indicates the presence of an active illness process. They observed urinary casts in 31 per cent of dogs with ARF, and out of those dogs, 80 per cent had fewer than 5 urinary casts per low power field.

Glucosuria was observed in 23-42 per cent of animals suffering from acute renal damage (Hokamp et al., 2016).

Urine culture is considered as the gold standard criteria for early diagnosis of lower urinary tract infections. Common isolates included *E coli* followed by gram positive cocci (Lulich & Osborne, 2004).

#### **2.9.4 Serum biomarkers**

Creatinine is regarded as the major biomarker in RIFLE renal failure staging. To determine AKI grades, a modified IRIS classification is used, which is based on serum creatinine, urine output and the need for renal replacement therapies (McNamara et al., 1989).

Cystatin C permeates through glomerular pores and readily absorbed in proximal tubular cells, with negligible tubular secretion. Thus Cystatin C can be a promising marker for PCT injury (Grubb, 2001).

Grauer (2005) stated that SDMA was more sensitive than creatinine, aids in the early detection and thus treatment of renal illness. Increase in the levels of serum SDMA ( $>14 \mu\text{g/dL}$ ) with isosthenuric urine, normal renal echotexture and serum creatinine ( $>1.4 \text{ mg/dL}$ ) was an indication of kidney injury in dogs.

SDMA remains stable in healthy dogs, but rise with disease progression in sick dogs having renal impairment, associating substantially with an increase in serum creatinine and thus decrease in GFR values (Nabity et al., 2015).

Kielstein et al. (2006) indicated that ADMA and SDMA were specific markers of GFR and are good predictors of early renal failure compared to serum creatinine levels, and thus were considered promising biomarkers for the early identification of renal dysfunction in dogs.

NGAL is a potential biomarkers for the early detection of tissue injury associated with various toxicities due to its higher sensitivity in case of early renal injuries (Zhou et al., 2014) .

### 2.9.5 Urine biomarkers

Urinary biomarkers are considered as a unique method for detecting acute renal damage in dogs. They are employed to diagnose renal impairment as early as possible (without evident signs) because the sensitivity and specificity of renal illnesses are altered by both renal and non-renal variables (Segev et al., 2015).

Pfleiderer et al. (1980) observed that enhanced urinary activity of alkaline phosphatase (a brush-border enzyme) has been linked to the proximal tubular injury in dogs.

Demars et al. (1989) reported that 1-microglobulin is found in extremely modest quantities in normal urine. Reabsorption of 1-microglobulin is inhibited under conditions of disrupted tubular function, and higher quantities are observed in urine in tubular insufficiencies.

Schnellman (2001) observed urinary ALKP, LDH, GGT and NAG as earliest marker of injury than proteinuria.

Raila et al. (2010) reported that microalbuminuria has a controversial role in the early detection of renal impairment. The finding of microalbuminuria in non-renal illnesses raises the question of the condition's specificity for the diagnosis of renal illness. Moreover, the presence of urine albumin is not a location-specific. It can be caused by both glomerular and tubular dysfunction, while severe albuminuria is usually linked with the glomerular problems.

Haase et al. (2011) reported that NGAL is a neutrophil-derived protein whose concentration in plasma and urine rises as the renal injury progresses. Plasma NGAL may be useful in distinguishing AKI from CKD in dogs with renal azotemia, particularly when the threshold value of >37.4 ng/mL is used (sensitivity 65% and specificity 82%).

The urinary cystatin C to creatinine ratio was found to be a promising measure for early assessment of the renal tubular dysfunction in dogs (Sasaki et al., 2014).

Urinary Alkaline phosphatase and SDMA activity considerably increases in PCT lesion and positively correlated with reduced GFR in dogs (Raekallio et al., 2006)

Under normal circumstances, only traces of cystatin C can be identified in conclusive urine. Urinary cystatin C levels rises with proximal tubular injury. Total

proteinuria must be measured since significant proteinuria has been demonstrated to block tubular reabsorption of cystatin C, resulting in increased urine cystatin C concentrations (Kovarikova, 2015).

Enhanced levels of NGAL in urine can be either renal or extra renal i.e., lower urinary tract or in drug induced nephrotoxicity (Cianciolo et al., 2016).

Konwar et al. (2017) stated that increased urine  $\gamma$ -glutamyl Trans peptidase activity is often used to identify the acute kidney damage.

KIM-1 (Kidney injury molecule-1) and GGT (Gamma glutamyl transferase) have been identified as excellent indicators for the early detection of AKI. KIM-1/uCr values in urine were much greater in AKI dogs compared to healthy dogs. Urine KIM-1 levels rose earlier in stage 1 (non-azotemic AKI) than in advanced AKI (Lippi et al., 2018).

## **2.9.6 Diagnostic imaging**

### **2.9.6.1 Ultrasonography**

Ultrasonography examination is an important part of a full evaluation of the urinary system functioning.

When specialized silver stain was used, leptospiral organisms were found in the renal tubules of two dogs under study. The medullary band was visible on ultrasound as a dark red to purple ring in the medulla. Congestion, bleeding, necrosis, and oedema were also observed on histology (Forrest et al., 1998).

Haller and Hizoh (2004) noted that the cortex is generally hyperechoic as compared to the more interior hypoechoic medulla in comparison to the liver. The cortex is normally isoechoic to hypoechoic and to the spleen it is hypoechoic.

The medullary rim sign has been linked to a number of pathological alterations in dogs, including hypercalcaemic nephropathy, end-stage renal disease, renal tubular necrosis and feline infectious peritonitis in cats. Acute tubular necrosis may cause a medullary rim sign early in the course of the toxicosis (Widmer et al., 2004).

Detection (2010) stated that ultrasonography can detect various anomalies in the renal pelvis, renal pelvic diverticula and ureters early, obviating the need for excretory urography.

Ultrasound findings in canines and felines with AKI were characterised by expanded renal size, expanded cortical echogenicity, perirenal liquid, pyelectasia,

expanded echogenicity of the perirenal fat and abnormal reverberation of urine (Mannucci et al., 2019).

An ultrasound examination of a patient suffering from acute kidney damage may reveal normal or enlarged kidneys, hyper echogenic renal cortices, or sub capsular oedema (Spatola & Andrulli, 2016).

Abdominal ultrasonography abnormalities were found in 85 per cent of dogs with acute grape toxicity, characterised by morphological changes in the kidneys including renomegaly, hyperechoic renal cortices and pelvic dilatation (Eubig et al., 2005).

#### **2.9.6.2 Radiography**

The irregularities in the shape and size of the kidneys and urine bladder, as well as other localised anomalies such as stones, growths/space occupying lesions, and obstruction of the entire urinary system can be outlined using routine radiographic examination (Essman, 2005).

Kumar et al. (2011) in a study on 10 dogs with azotemia, observed distended urinary bladder, irregular radiopaque densities in the renal pelvis and radiopaque calculi.

#### **2.9.7 Electrocardiography**

Langston (2008) reported that cardiac abnormalities can manifest themselves in a variety of ways, including ventricular and supraventricular arrhythmias. U waves appear on the ECG only in a small percentage of cases of ARF.

Rusenov et al. (2014) observed a steady decrease in the amplitudes of the P and R peaks, as well as an enhanced and sharp-edged repolarization of T wave in acute renal injury caused by ethylene glycol intoxication.

### **2.10 Treatment and management**

The major goal of treatment for uremia is to address changes in the patient's internal state. Ideal treatment should allow the patient to live long enough for renal damage to be repaired in an acute injury and hypertrophy of viable remnant nephrons to occur in a chronic injury, resulting in better renal function and the capacity to lead a normal life.

Managing AKI includes two primary strategies: optimizing renal perfusion and regulation of intrarenal pathophysiological instruments of AKI and includes

intravenous fluids, vasopressors and diuretics (Gonsalez et al., 2019). A polyionic fluid such as lactated Ringer's solution is satisfactory unless hyperkalemia is present, in which case normal saline is recommended. Sodium bicarbonate may be added to correct acidosis. Fluid therapy and diuretics were directed according to the clinical signs and the actual assessment of dehydration status with emphasis in promoting diuresis and promoting renal hemodynamics.

### **2.10.1 Conventional therapy**

The injury process can be controlled by correcting the metabolic consequences of acute renal failure, or the injury process can be inhibited by accelerating the repair process of body.

Volume overload, solute overload (hyperkalemia acidosis, uremia and cytokines), endocrine deficits (erythropoietin) and non-renal problems (sepsis, GI bleeding, disorientation, respiratory failure) are all potential impacts of renal failure (Mahony, 1976).

Balanced polyionic solutions are considered to be appropriate for initial resuscitation and replacement of the dehydration deficit. After rehydration, maintenance fluids with lower  $\text{Na}^+$  are more appropriate. Colloids can be indicated in hypoalbuminemia (Langston, 2008).

Mannitol can be used to improve intravascular volume and tubular flow, as well as to avoid tubular obstruction before ischemia or toxic shock occurs. Mannitol also has modest vasodilatory effect and, if given early in ARF, may enhance renal blood flow and thus improves glomerular filtration (Finn, 1990). He further reported that dopamine is a selective renal vasodilator that increases urine production and induces significant natriuresis, if used early in ARF. Low-dose dopamine (0.5-3 g/kg/min) stimulates renal D1, D2, and D4 receptors, increasing renal blood flow and promoting natriuresis. Low-dose dopamine causes an increase in glomerular filtration rate in normal dogs. Low-dose dopamine, also known as renal-dose dopamine, has long been regarded as Reno protective.

Furosemide is a loop diuretic and a vasodilator that reduces the metabolic work of the thick ascending limb while also flushing obstructive casts from the nephron. It may also lower the concentration of toxins in the tubules, such as

myoglobin or haemoglobin. Furosemide may convert oliguric ARF to non-oliguric ARF (Shilliday & Allison, 1994).

The retention of water and electrolytes is reasonable in oliguric renal dysfunction, while loss of water and electrolytes is the principle worry in non-oliguric ARF. Treatment with fluids re-establish the liquid and electrolyte balance, increment renal hemodynamic and advances diuresis as part of ARF treatment (Grauer, 1996).

Lulich and Osborne (2004) mentioned that antibiotic therapy should be based on the results of susceptibility testing. Antibiotics with a high renal excretion rate are preferred because they ensure therapeutically effective drug levels in the urine.

As observed by Labato (2001), doxycycline should be used in hemoprotozoan or rickettsial disorders, however ampicillin or enrofloxacin can be used till confirmatory diagnosis were made.

Rajapakse and Wijewickrama (2009) observed that while dialysis obviously improves outcomes in sepsis-induced acute renal damage, numerous alternative therapies are also beneficial. Particularly beneficial are cautious hydration volume loading, the use of vasopressors inotropes to maintain mean arterial pressure and the avoidance of ionic contrast agents and nephrotoxic medications.

Antihypertensive regimens that include ACE inhibitors are more effective than regimens that do not include ACE inhibitors in reducing the progression of nondiabetic kidney disease (Roudebush et al., 2010).

### **2.10.2 Intermittent Hemodialysis**

Intermittent hemodialysis (IHD) is basically based on Fick's law in which solute transfer occurs on gradient basis. Convective and diffusion removal followed by ultrafiltration are sequenced in each cycle (Misra, 2008).

Peritoneal dialysis or hemodialysis may be necessary if none of conventional measures restores urine production (Brown et al., 2013).

IHD is an extracorporeal renal replacement therapy and is most common indication for acute kidney injury (AKI). Based on principle of diffusion, therapeutic role of hemodialysis is to remove uremia toxins and solute overload from the body and alleviate the morbidity and clinical features (Cowgill, 2011).

ARF is the most common indication for dialysis in veterinary medicine. Hemodialysis has been used to successfully treat clinical manifestations of uremia in

dogs (Segev et al., 2008). Patients with severe uremia who receive hemodialysis have a longer life expectancy and a greater window of opportunity for recovery.

Pelander et al. (2015) stated that refractory azotemia (BUN > 90 mg/dl, serum creatinine > 6 mg/dl), acute renal insult, hyperkalemia, fluid overload, severe metabolic acidosis, acute poisoning or drug overdose, preoperative conditioning for renal transplantation and post-operative delayed graft function are all indications for veterinary hemodialysis.

When serum urea nitrogen concentrations surpass 90 mg/dl (32 mmol/l) and serum creatinine concentrations exceed 8 mg/dl (700 mol/l), hemodialysis is most likely to be effective (Segev et al., 2016).

Segev et al. (2008) concluded hemodialysis to be effective in initial stages of AKD with minimal renal damage. Severity or progression towards chronic stage tags IHD just a life prolongation step with poor prognosis. However, with the etiology than can be managed medically, conventional therapy as well as IHD had almost similar outcomes.

## CHAPTER III

### MATERIALS AND METHODS

#### 3.1 Place of the study

The present study entitled “A study on early diagnosis of acute kidney disease and its therapeutic management in dogs” was undertaken at Department of Veterinary Medicine and Small Animal Clinics, Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana.

#### 3.2 Criteria for the selection of Clinical cases and Experimental Design

The present study has been planned with the objectives: (i) To undertake early diagnosis of acute kidney disease in dogs. (ii) To evaluate treatment with or without hemodialysis in acute kidney disease in dogs.

**Experiment I:** To undertake early diagnosis of acute kidney disease in dogs.

**Group I (Apparently healthy dogs):** Twenty apparently healthy dogs presented at Small Animals Clinics of Teaching Veterinary Clinical Complex, GADVASU, Ludhiana for routine examination, deworming, vaccination and general health check up with apparently normal physical examination were selected as a control group.

**Table 1: Age wise distribution of healthy dogs (n=20)**

| Age group  | Total (n=20) |
|------------|--------------|
| < 4 years  | 11           |
| 4-8 years  | 7            |
| 8-10 years | 2            |

#### **Group II (Acute Kidney Disease –AKD dogs):**

Dogs presented with a history of general illness, anorexia, vomiting, oliguria or anuria of  $\leq 7$  days presented at Small Animal Clinics GADVASU, Ludhiana were selected for further detailed examination. Out of these 30 dogs of renal insufficiency/failure (BUN > 25 mg/dL and Cr > 1.6 mg/dL) indicative of Acute Kidney Diseases were selected and were classified as per IRIS guidelines.

#### 3.3 Observations recorded

##### 3.3.1 Owner information

Name, address and phone no. of the owner was recorded in case records.

### 3.3.2 Signalment

Patient specific data i.e. age, breed, sex and weight were recorded.

**Table 2: Age wise distribution of dogs with acute kidney disease (n=30)**

| Age group  | Total               |
|------------|---------------------|
| 6m-2 years | 3 (10) <sup>1</sup> |
| 2-4 years  | 12 (40)             |
| 4-8 years  | 12 (40)             |
| 8-12 years | 3 (10)              |

<sup>1</sup>Figures in parentheses indicate percentage of dogs

**Table 3: Gender wise distribution of dogs with acute kidney disease (n=30)**

| Sex    | No. of animals         |
|--------|------------------------|
| Male   | 19 (63.3) <sup>1</sup> |
| Female | 11 (36.6)              |

<sup>1</sup>Figures in parentheses indicates the percentage of animals

### 3.3.3 Patient history

A brief anamnesis of apparently healthy and AKD dogs w.r.t weight loss, reduced appetite, change in water intake (polydipsia), stools (consistency, colour and frequency), urination (colour, posture, frequency- polyuria/oliguria/anuria), vaccination & deworming history, suspicious poisoning or ingestion of noxious substance, vomition (colour, frequency and contents), fever, ticks infestation, exercise intolerance, concurrent urinary tract infections, uroliths, days of onset of clinical signs, any previous illness and treatment provided.

Only those dogs which were having normal clinical history were included in healthy group, while other dogs were subjected for detailed physical examination and different diagnostic tests (Hematology, biochemistry, urinalysis, hemoprotozoan examination, radio-diagnostics, etc.) to identify the cause of the disease.

### 3.3.4 Physical examination

A complete clinical examination was performed in apparently healthy and AKD dogs. Recording vitals like rectal temperature, respiration rate, heart rate; auscultation of the lungs & heart; examination of conjunctiva, oral cavity for dental tartar & ulcer or any necrosis; gross skin coat examination for any parasitic

infestation, dehydration status; abdominal palpation to rule out pain or any growth & regional lymph node. Based on the above observations dogs, with normal parameters were included in apparently healthy group and those with abnormalities were included in disease group. The disease group animals were subjected to the further evaluation for diagnosis of kidney disease.

### **3.4 Hemato-biochemical analysis**

#### **3.4.1 Blood collection**

Following proper casting/restrain of animal, about 2-3ml blood was collected from either cephalic or saphenous vein in Na<sub>2</sub>EDTA vials for CBC, fibrinogen estimation and examination of hemoprotozoan. Five ml of blood was collected in clot activated vials which were later centrifuged to extract the serum. Serum was stored at -20°C for further analysis of various biochemical parameters.

#### **3.4.2 Haematology**

Hematology analysis was done on laser based fully automated (ADVIA 2120 Hematology system, Siemens Healthcare and Diagnostics Inc., USA). Hematological parameters included were Hb (g/dl), PCV (packed cell volume, %), TLC ( $10^3/\text{mm}^3$ ), platelet count ( $10^3/\text{mm}^3$ ), TEC ( $10^6/\text{mm}^3$ ), absolute neutrophil count ( $/\text{mm}^3$ ), absolute lymphocyte count ( $/\text{mm}^3$ ). After preparing blood smear on a clear glass slide, staining with Leishman's stain was done, Differential leukocyte count (DLC) was performed under oil immersion lens.

#### **3.4.3 Blood biochemical analysis**

Fully automatic Chemistry analyser (Ortho Clinical Diagnostics, Johnson & Johnson, Co.) was used for the analysis of following biochemical parameters using Vitrose DT slides:

- a) Renal function parameters:** (i) Serum creatinine (mg/dl), (ii) BUN (mg/dl)
- b) Liver function parameters:** (i) ALT (U/L), (ii) AST (U/L), (iii) GGT (U/L), (iv) ALKP (U/L), (v) Total bilirubin (TB, mg/dL), (vi) Total protein (TP, g/dL), (vii) Albumin (g/dL), (viii) Blood glucose (mg/dL) and (ix) Cholesterol (mg/dL).
- c) Minerals/ Electrolytes:** (i) Na<sup>+</sup> (mEq/L) (ii) K<sup>+</sup> (mEq/L) (iii) Cl<sup>-</sup> (mEq/L) (iv) Ca (mg/dL) (v) P<sub>i</sub> (mg/dL) (vi) Mg (mg/dL).
- d) Urine protein (mg/dL) and Urine uric acid (mg/dL).**

#### 3.4.4 Fibrinogen estimation

For plasma fibrinogen estimation, heat precipitation method was used. Fresh blood in anticoagulant vials was transferred to two microhematocrit tubes. Both tubes were sealed at one end by sealant and centrifuged at 14000 rpm for about 3 minutes. One tube was taken out and was broken manually at the RBC/plasma interface, plasma was poured onto the prism of the refractometer, and the reading was noted. The other tube was placed in a hot water bath (58°C) for about 3 minutes and later centrifuged at the same speed for the about 3 minutes. The tube was broken at the RBC/plasma contact and the protein concentration of the residual plasma was measured. The plasma fibrinogen value was calculated from difference between 1<sup>st</sup> and 2<sup>nd</sup> readings.

#### 3.4.5 Renal and Cardiac biomarkers

Following renal and cardiac biomarkers were estimated using ELISA kits (Biocodon™ Technologies, USA):

- (i) **Serum renal biomarkers:** SDMA (Symmetric Dimethyl Arginine, µg/dL), NGAL (Neutrophil Gelatinase-Associated Lipocalin, ng/mL) and Cystatin C (mg/L).
- (ii) **Serum cardiac biomarker:** cT-I (Cardiac troponin I) (ng/dL).
- (iii) **Urine renal biomarkers:** SDMA (µg/dL), NGAL (ng/mL), Cystatin C (mg/L).

#### Assay criteria of ELISA

1. Prerequisite for the assay includes maintaining normal room temperature conditions.
2. Number of strips needed varies and thus put in frame accordingly.
3. A standard diluent of 100 µl was put in blank wells.
4. 50µl of standard solution and 50µl streptavidin-HRP were added to the standard wells.
5. 40µl of undiluted sample, 10µl anti- antibodies and 50 µl of streptavidin-HRP were added to blank well.
6. Mixture was thoroughly agitated in well and the plate was then covered with a sealer and placed in incubator at 37°C for about 60 minutes.
7. Contents of all the wells were aspirated, discarded and plate wells were rinsed 5 times with the wash buffer then blotted the plate onto paper or any suitable absorbent material.

8. 50µl substrate solution A was added to each well followed by addition of 50µl solution B. After gentle shaking and covering the well with sealer, plate was kept in incubator at 37°C in the dark room for about 10 minutes.
9. A stop solution of 50µl was added to each well and mixed properly. The blue colour of solution changed into yellow immediately after addition.
10. Taking blank well as zero, absorbance was measured at 450nm wavelength.

**Result interpretation:**

1. A standard curve was drawn by plotting the mean absorbance of each reference standard (OD), against its concentration with absorbance on y-axis and concentration on x-axis.
2. Calculate the linear regression equation of standard curve according to the standard concentration and the OD value.

**3.4.6 Blood parasitological examination**

**3.4.6.1 Blood smear examination**

Blood smears from dogs suspected for AKD were prepared on glass slides, stained with Leishman stain and examined under the microscope for presence of hemoprotozoa and rickettsia.

**3.4.6.2 Molecular technique**

Blood samples taken from AKD suspected dogs were subjected to DNA isolation using polymerase chain reaction (PCR) for diagnosing various hemoprotozoans and rickettsia. After standardization of a given parasite with specific PCR assays, Their DNA amplicons were observed in horizontal agarose gel (1.5%) electrophoresis at 672 bp (*B. gibsoni*), 601 bp (*B. vogeli*), 380 bp (*E. canis*) and 666 bp (*H. canis*).

**3.4.6.2.1 DNA isolation of hemoprotozoa using Polymerase Chain Reaction**

Genomic DNA extraction was done from the blood collected in EDTA. QIAamp DNA mini blood kit (Qiagen, Germany) was used as per manufacturer's protocol with only exception that the final dilution was done in a 100 µl volume. The procedure followed has been given in Plate 3.

**3.4.6.2.2 *Babesia gibsoni* PCR Standardization:**

The *Babesia gibsoni* PCR technique as per Singh et al. (2014) was standardized by using the primers, targeting its partial 18S rRNA. Specific genomic patterns used were:

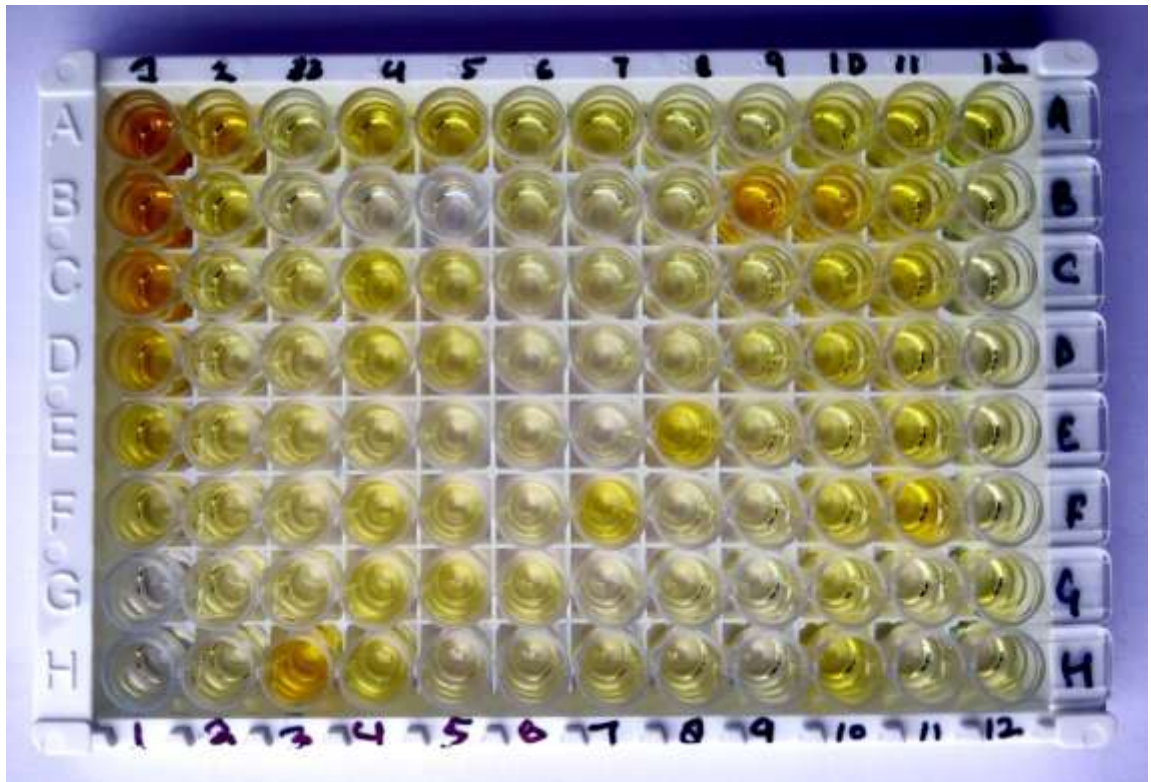


Plate 1: ELISA microtiter plate

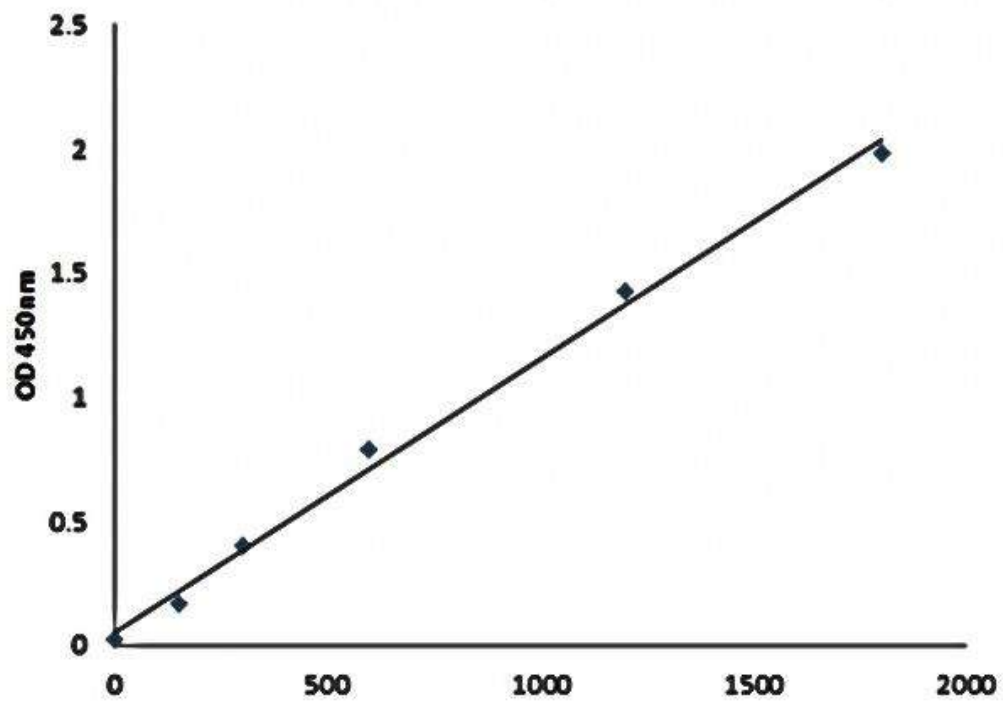


Plate 2: Standard curve of ELISA

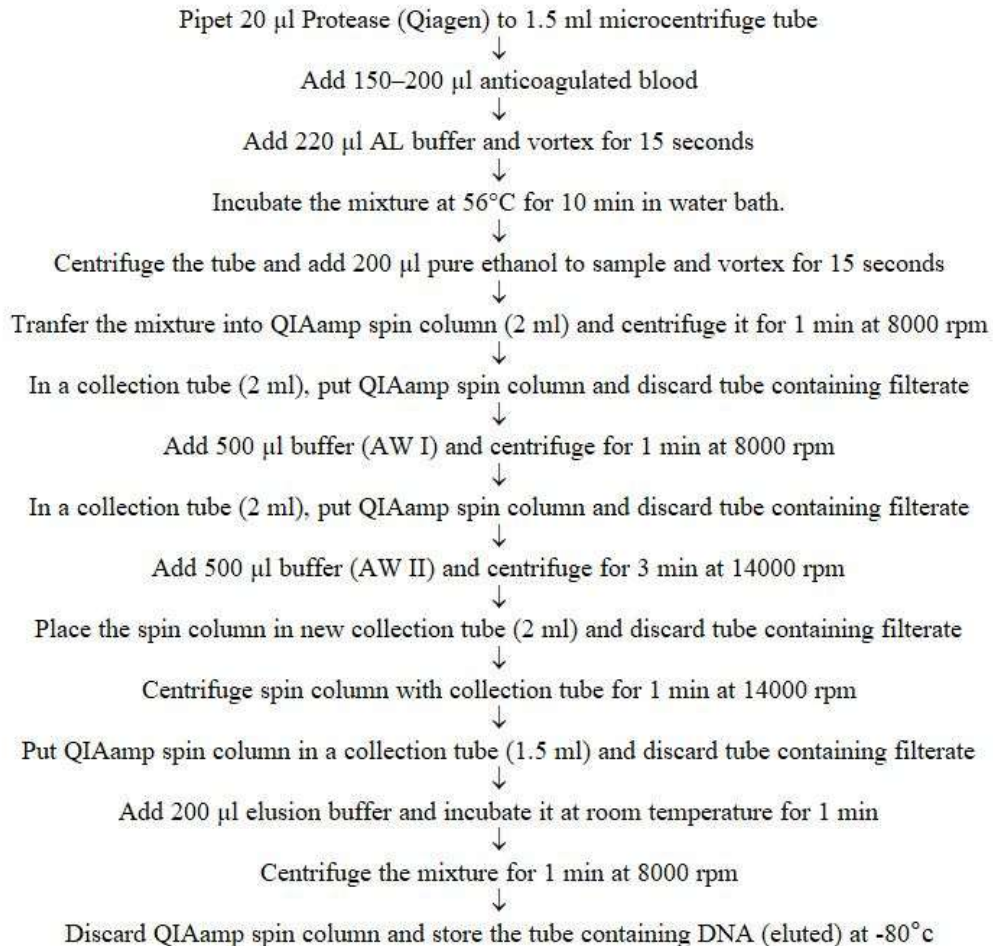


**Plate 3: Flowchart of DNA isolation procedure**

Forward primer: 5' CTC GGC TAC TTG CCT TGT C 3'

Reverse primer: 5' GCC GAA ACT GAA ATA ACG GC 3'

The components of PCR in a final volume of 25 µl are given in table below:

**Table 4: Composition of master mix for PCR assay of *B. gibsoni***

| S No.      | Components                                    | Quantity (µl) |
|------------|-----------------------------------------------|---------------|
| 1.         | Dream buffer (10X) + MgCl <sub>2</sub> (20mM) | 2.5           |
| 2.         | dNTP mixture (10mM)                           | 0.5           |
| 3.         | Magnesium chloride (25mM)                     | 0.5           |
| 4.         | Forward Primer (15 pmol GIB599)               | 1.0           |
| 5.         | Reverse Primer (15 pmol GIB1270)              | 1.0           |
| 6.         | <i>Taq</i> Polymerase (1.0 U)                 | 0.25          |
| 7.         | Sample (3.0 µl)                               | 3.0           |
| 8.         | NFW                                           | 16.25         |
| Net volume |                                               | 25.0          |

The tube was then spun gently and placed in a thermocycler.

#### **3.4.6.2.3 *Babesia vogeli* PCR Standardization:**

The *Babesia vogeli* PCR technique as per Duarte et al. (2008) was standardised by using the primers, targeting its partial 18S rRNA. Specific genomic patterns used were:

Forward primer: 5' GTG AAC CTT ATC ACT TAA AGG 3'

Reverse primer: 5' CAA CTC CTC CAC GCA ATC G 3'

The components of PCR in a final volume of 25 µl are given in table below:

**Table 5: Composition of master mix for PCR assay of *B. vogeli canis***

| S No.      | Components                                    | Quantity (µl) |
|------------|-----------------------------------------------|---------------|
| 1.         | Dream buffer (10X) + MgCl <sub>2</sub> (20mM) | 2.5           |
| 2.         | dNTP mixture (10mM)                           | 0.5           |
| 3.         | Magnesium chloride (25mM)                     | 0.5           |
| 4.         | Forward Primer (15 pmol BAB1)                 | 1.5           |
| 5.         | Reverse Primer (15 pmol BAB4)                 | 1.5           |
| 6.         | <i>Taq</i> Polymerase (1.0 U)                 | 0.25          |
| 7.         | Sample (3.0 µl)                               | 3.0           |
| 8.         | NFW                                           | 15.75         |
| Net volume |                                               | 25.0          |

The tube was then spun gently and placed in a thermocycler.

#### 3.4.6.2.4 *Hepatozoon canis* PCR Standardization:

The *Hepatozoon canis* PCR technique as per Inokuma et al. (2002) was standardised by using the primers, targeting its partial 18S rRNA. Specific genomic patterns used were:

Forward primer: 5' ATA CAT GAG CAA AAT CTC AAC 3'

Reverse primer: 5' CTT ATT ATT CCA TGC TGC AG 3'

The components of PCR in a final volume of 25 µl are given in table below:

**Table 6: Composition of master mix for PCR assay of *H. canis***

| S. No.     | Components                                    | Quantity (µl) |
|------------|-----------------------------------------------|---------------|
| 1.         | Dream buffer (10X) + MgCl <sub>2</sub> (20mM) | 2.5           |
| 2.         | dNTP mixture (10mM)                           | 0.5           |
| 3.         | Magnesium chloride (25mM)                     | 0.5           |
| 4.         | Forward Primer (15 pmol HEP-F)                | 1.0           |
| 5.         | Reverse Primer (15 pmol HEP-R)                | 1.0           |
| 6.         | <i>Taq</i> Polymerase (1.0 U)                 | 0.25          |
| 7.         | Sample (3.0 µl)                               | 3.0           |
| 8.         | NFW                                           | 16.75         |
| Net volume |                                               | 25.0          |

The tube was then spinned gently and placed in a thermocycler.

#### 3.4.6.2.5 *Ehrlichia canis* PCR Standardization:

The *Ehrlichia canis* PCR technique as per Kledmanee et al. (2009) was standardized by using the primers, targeting its partial Vir B9 gene. Specific genomic patterns used were:

Forward primer: 5' CCA TAA GCA TAG CTG ATA ACC CTG TTA CAA 3'

Reverse primer: 5' TGG ATA ATA AAA CCG TAC TAT GTA TGC TAG 3'

The components of PCR in a final volume of 25 µl are given in table below:

**Table 7: Composition of master mix for PCR assay of *E. canis***

| S No.      | Components                                    | Quantity (µl) |
|------------|-----------------------------------------------|---------------|
| 1.         | Dream buffer (10X) + MgCl <sub>2</sub> (20mM) | 2.5           |
| 2.         | dNTP mixture (10mM)                           | 0.5           |
| 3.         | Magnesium chloride (25mM)                     | 0.5           |
| 4.         | Forward Primer (15 pmol Ehr1401F)             | 1.0           |
| 5.         | Reverse Primer (15 pmol Ehr1780R)             | 1.0           |
| 6.         | <i>Taq</i> Polymerase (1.0 U)                 | 0.25          |
| 7.         | Sample (3.0 µl)                               | 3.0           |
| 8.         | NFW                                           | 16.75         |
| Net volume |                                               | 25.0          |

The tube was then spinned gently and placed in a thermocycler.

#### **3.4.6.2.6 Detection of amplified products of PCR**

Amplicons of PCR were tested for electrophoresis amplification with agarose gel (1.5%) with nucleic acid stain (Helix Biosciences) for 45 minutes at 100 volts current. For achieving concentration of 0.5 µg/ml, nucleic acid stain (5 µL) was added and cooled at room temperature. The gel formed was put in horizontal gel electrophoresis system with TAE (1X) buffer. The samples of DNA were put in wells and PCR amplified products were combined with Tri Track DNA loading dye (3 µL of 6X). The apparatus was run at 100 volts for 45 minutes. The amplicons were then visualized in Bio-Rad gel documentation system and amplicons sizes were calculated. DNA isolated from control dogs were negative for hemoprotozoa and samples of clinically infected dogs found to be positive for hemoprotozoa and rickettsia on Giemsa stain served as positive control.

**Table 8: Cycling conditions of PCR for different hemoprotozoans and rickettsia**

| <b>Blood protozoa</b>                       | <b>Target</b> | <b>Size of Product</b> | <b>Cycling conditions (Temp/time)</b> | <b>Cycles</b> |
|---------------------------------------------|---------------|------------------------|---------------------------------------|---------------|
| <i>B. gibsoni</i><br>(Singh et al., 2014)   | 18S           | 672 bp                 | Starting denaturation (95°C /5 min)   | 40            |
|                                             |               |                        | Denaturation (95°C /30 sec)           |               |
|                                             |               |                        | Annealing (62°C /30 sec)              |               |
|                                             |               |                        | Extension (72°C / 45 sec)             |               |
|                                             |               |                        | Final extension (72°C/ 7 min)         |               |
| <i>B. vogeli</i><br>(Duarte et al., 2008)   | 18S           | 601 bp                 | Starting denaturation (95°C /5 min)   | 40            |
|                                             |               |                        | Denaturation (95°C /30 sec)           |               |
|                                             |               |                        | Annealing (60°C /30 sec)              |               |
|                                             |               |                        | Extension (72°C / 45 sec)             |               |
|                                             |               |                        | Final extension (72°C/ 7 min)         |               |
| <i>H. canis</i><br>(Inokuma et al., 2002)   | 18S           | 666 bp                 | Starting denaturation (95°C /5 min)   | 40            |
|                                             |               |                        | Denaturation (95°C /30 sec)           |               |
|                                             |               |                        | Annealing (58°C/ 30 sec)              |               |
|                                             |               |                        | Extension (72°C / 45 sec)             |               |
|                                             |               |                        | Final extension (72°C/ 7 min)         |               |
| <i>E. canis</i><br>(Kledmanee et al., 2009) | VirB9         | 380 bp                 | Starting denaturation (95°C /5 min)   | 40            |
|                                             |               |                        | Denaturation (95°C /30 sec)           |               |
|                                             |               |                        | Annealing (62 °C/ 30 sec)             |               |
|                                             |               |                        | Extension (72°C / 45 sec)             |               |
|                                             |               |                        | Final extension (72°C/ 7 min)         |               |

### 3.5 Blood pressure measurement

The VET-HDO-MONITOR-NIBP-SYSTEM which works on the concept of high resolution oscillometry was used to measure the blood pressure of dogs. A cuff was wrapped around the patient's limb, aerated-flattened and so on, measuring SAP, DAP and MAP.

The instrument includes Doppler unit and sphygmomanometer, ultrasound coupling gel and inflatable cuff (width should be 40% of the limb circumference). The

animal was placed in lateral recumbency and once the artery has been located, probe was held still and cuff was inflated until the ‘whooshing’ sound of the pulse was no longer heard. Gradually, the cuff was deflated using the pressure release button/valve and listened for the return of the ‘whooshing’ sound to evaluate Mean, Systolic and Diastolic Arterial Pressure, respectively.

### **3.6 Electrocardiography**

Electrocardiogram (ECG) was recorded using Bailey’s hexaxial limb lead I, II, III, aVR, aVL and aVF. Lead II was used to interpret ECG readings. The ECG readings were usually taken at speed of 50mm per sec with the help of various leads (Tilley, 2008).

### **3.7 Urine analysis**

The urine samples were collected in a sterile plastic container preferably via cystocentesis or urinary catheter or 1<sup>st</sup> morning sample of urine collected by owner (natural voided).

#### **3.7.1 Physical examination**

Urine samples collected were examined for colour, smell, turbidity, sediments, etc. Urine specific gravity was measured using refractometer. The device was being standardised using distilled water to ‘1.000’ reference value. A urine drop was then placed on stage and value was noted.

#### **3.7.2 Qualitative urine biochemical analysis**

Urine samples were analyzed qualitatively for ketone bodies (KET), nitrite (NIT), protein (PRO), glucose (GLU), blood (BLD), leukocytes (LEU), urobilinogen (URO), bilirubin (BIL) and pH using SIEMENS Multistix 10 SG. The readings were noted on ORINALYSER U 120 analyser.

#### **3.7.3 Quantitative urine biochemical analysis**

Urine samples were centrifuged and supernatant were analyzed for urine protein, urine creatinine, GGT, ALP and uric acid with the help of Vitros DT-350 chemistry analyzer.

#### **3.7.4 Urine microscopic examination**

For microscopic examination, urine samples were centrifuged at 2,000 rpm for about 5 minutes and the supernatant was discarded. Sediment was again re-suspended

in urine. Its one drop was put on glass slide and examined both under low and high power of microscope and examined for various casts, cells, crystals, etc.

### **3.7.5 Urine culture and CST**

Urine samples collected via cystocentesis (uncentrifuged) were taken in a sterile vial and examined for any viable growth. A loop with urine sample was streaked on blood agar and incubated at 37°C for 24 hours and later observed for any colony growth, and its morphology.

For identification of bacteria, colony growth was heat fixed on a glass slide and then stained with Gram's stain. The slide was examined under the oil immersion to identify the bacterial morphology.

For identification of a particular bacterium, specific media was used. A loop of colony was taken from growth and streaked on selective media i.e. Baird Parker Agar for *Staphylococcus*; Eosin Methylene Blue Agar for *E. coli* and Mannitol Salt Agar for *Streptococcus* species.

Colonies identified were then streaked on nutrient agar for antibiotic sensitivity testing using specific antibiotic disc pads. Inhibition zones for various antibiotics were measured.

## **3.8 Imaging**

### **3.8.1 Radiography**

Some of the suspected cases of AKD were subjected to X-ray examination for chest (lateral, VD view) to rule out any cardiomegaly, pleural effusions, etc. and abdomen to view liver, kidneys, prostate, urinary bladder and urethra for any suspected mass, growth, nephrolith, cystolith or urethrolith.

### **3.8.2 Ultrasonography**

Some of the suspected cases of AKD were subjected to abdominal ultrasonography was performed by teachers of Department of Veterinary Surgery and Radiology. B mode of Philips Affinity 70 ultrasound machine was used. The findings of ultrasonography related to kidneys, ureters, urinary bladder, intestines, liver, spleen and prostate or uterus were noted.

## **3.9 Management of Acute Kidney Disease cases**

**Experiment II:** To evaluate treatment with or without hemodialysis in acute kidney disease in dogs, therapeutic management of dogs (n=30) with Acute Kidney Disease

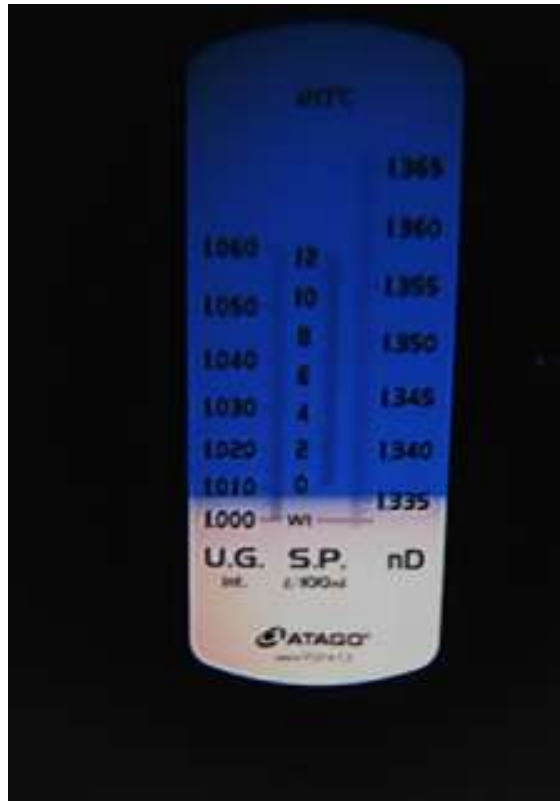


**Plate 4: Refractometer reading of urine specific gravity**

was undertaken with conventional treatment and hemodialysis as per suspected etiology and hematobiochemical findings:

- a) Conventional treatment included mainly fluid therapy, diuretics and/ ACE inhibitors, symptomatic treatment, etc., as per requirement (n=26).
- b) Intermittent hemodialysis along with conventional treatment (n=4).

All patients were provided symptomatic treatment initially to ameliorate clinical signs of any electrolytic disturbances, uraemia, provided vitamin and mineral support along with dietary modifications. Treatment regimen included fluid therapy, antibiotics, diuretics, gut probiotics, Reno protectants and mineral supplementation, etc.

For intermittent hemodialysis, Fresenius 4008S machine having features of online clearance monitoring (OCM) was used. IHD was performed in 4 AKD dogs of which three dogs received only single session of hemodialysis and 1 dog received 3 sessions of dialysis. The detailed conventional treatment is mentioned in table below:

**Table 9: Treatment protocol for dogs with acute kidney disease (AKD) (n=30)**

| Drug                        | Dose rate                             |          | Route         | Indications                                                                            | No. of cases (n=30) |
|-----------------------------|---------------------------------------|----------|---------------|----------------------------------------------------------------------------------------|---------------------|
| RL+DNS (5%)                 | Dehydration volume                    |          | I/V           | Anorexia, Hypochloremia; Hyperkalemia Metabolic acidosis, Hyponatremia and Hypokalemia | 6                   |
|                             | 4-6 %                                 | 22 ml/kg |               |                                                                                        |                     |
| RL+N/2 DNS                  | 6-8 %                                 | 40 ml/kg |               |                                                                                        | 22                  |
|                             | 8-10 %                                | 80 ml/kg |               |                                                                                        |                     |
| RL                          | 10-12%                                | 100ml/kg |               |                                                                                        | 4                   |
| Mannitol                    | 0.25-1g/kg as a bolus over 20 minutes |          | I/V           | Osmotic Diuretic, Increases RBF, GFR, solute excretion and Tubular flow                | 4                   |
| Metoclopramide, Ondansetron | 0.2-0.5 mg/kg                         |          | I/M, S/C, I/V | Vomition                                                                               | 23                  |

| <b>Drug</b>     | <b>Dose rate</b>                  | <b>Route</b> | <b>Indications</b>                        | <b>No. of cases (n=30)</b> |
|-----------------|-----------------------------------|--------------|-------------------------------------------|----------------------------|
| Ranitidine      | 0.5-1 mg/kg                       | S/C, I/M     | Antacid                                   | 28                         |
| Furosemide      | 2-4 mg/ kg                        | I/V, I/M     | Oliguria, anuria                          | 27                         |
| Polybion        | 1ml/10 kg                         | I/M          | Vitamin supplementation                   | 26                         |
| Belamyl         | 1ml/10 kg                         | I/M          | Vitamin and Liver extract supplementation | 4                          |
| Metronidazole   | 10-15 mg/kg                       | I/V          | Neutrophilic leukocytosis                 | 4                          |
| Ampicillin      | 10mg/kg                           | I/M, I/V     |                                           | 15                         |
| Amikacin        | 5-10mg/kg                         | I/M, I/V     |                                           | 2                          |
| Enrofloxacin    | 5mg/kg                            | I/M, PO      |                                           | 3                          |
| Cefotaxime      | 20-25 mg/kg                       | I/M, I/V     |                                           | 1                          |
| Doxycycline     | 10mg/kg                           | PO           | Hemoprotozoan infection                   | 5                          |
| Tranexamic acid | 10 mg/kg                          | I/M          | Hemorrhagic enteritis                     | 3                          |
| Sucralfate      | 10 ml total dose                  | PO           | Gastro protectant                         | 5                          |
| Vitamin C       | 100-500 mg total dose             | I/V, PO      | HGE, urinary Acidifier                    | 3                          |
| RBC pet         | 10 ml, bid                        | PO           | Anemia                                    | 4                          |
| Platogrow       | 10 ml, bid                        | PO           | Thrombocytopenia                          | 5                          |
| Becosule        | 5ml, od                           | PO           | B-complex                                 | 2                          |
| Vitabest        | 10 ml, od                         | PO           | Omega 3, improve GFR                      | 22                         |
| Uripet          | 1 tsf for 10kg od                 | PO           | Urinary alkaliser                         | 2                          |
| Gut well        | 1 tsf for 10kg od                 | PO           | Gut dysbiosis                             | 25                         |
| Heckler         | 1 tab, bid (up to 10 kg)          | PO           | Reno protective                           | 30                         |
| Gelusil         | Al(OH) <sub>3</sub> , 40-100mg/kg | PO           | Hyperphosphatemia                         | 5                          |

### 3.10 Statistical analysis

The statistical analysis of the data was performed with help of t- test, one way ANOVA and Pearson's correlation using SPSS statistics. Post hoc Dunkens's test was used for determination of the significant difference among the means.

## CHAPTER – IV

### RESULTS AND DISCUSSION

The present study entitled **“A study on early diagnosis of acute kidney disease and its therapeutic management in dogs”** was undertaken at the Department of Veterinary Medicine and Small Animal Clinics, Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The study was conducted with the objectives: (i) To undertake early diagnosis of acute kidney disease in dogs; (ii) To evaluate treatment with or without hemodialysis in acute kidney disease in dogs.

In the first objective, dogs presented with renal insufficiency irrespective of sex, breed and age with signs of anorexia, inappetance, vomiting, oliguria, anuria, lethargy, melena and halitosis of  $\leq 7$  days duration were selected for the diagnosis of underlying renal insufficiency. Dogs with serum creatinine levels of  $\geq 1.6$  mg/dL and BUN  $\geq 25$  mg/dL were selected for further investigation. A total of 156 cases with above mentioned criteria were screened, out of which 77 cases were having Cr levels below 1.6 mg/dL and 49 cases were in CRF. So, 30 dogs of renal insufficiency/failure (BUN  $> 25$  mg/dL and Cr  $> 1.6$  mg/dL) which were having characteristics of Acute Kidney Disease were selected for further study and classified as per IRIS guidelines (Segev et al., 2016). IRIS Grade 1: non-azotemic AKI (serum creatinine  $< 1.6$  mg/dl), Grade 2: mild AKI (serum creatinine 1.7 to 2.5 mg/dl, n=10), Grade 3: moderate to severe azotemic (serum creatinine 2.6–5 mg/dl, n=12), Grade 4: moderate to severe azotemic (serum creatinine 5.1–10 mg/dl, n=7) and Grade 5: severe azotemic (serum creatinine  $> 10$  mg/dl, n=1).

Twenty apparently healthy dogs presented at Small Animals Clinics of Teaching Veterinary Clinical Complex, GADVASU, Ludhiana for routine examination, deworming, vaccination and general health check up with apparently normal physical examination findings were selected as a control group for comparative study.

Thirty cases of AKD were subjected to detailed investigations including history and physical examination. Blood and urine samples were collected and subjected to analysis of different parameters for hematology, biochemistry, routine

urinalysis, urine CST, renal biomarkers in serum and urine. They were subjected to hemoprotozoa and rickettsial examination by blood smear examination and PCR analysis. They were also subjected to diagnostic aids like radiology and ultrasonography, etc. as per requirement. Above investigations were undertaken to identify the cause of AKD.

The second objective was to undertake the treatment of these 30 cases of AKD with either conventional therapy alone (n=26) or combination of conventional and intermittent hemodialysis (n=4).

## 4.1 Signalment

### 4.1.1 Age

Mean age of the dogs affected with AKD was  $5.22 \pm 0.51$  years. The maximum dogs were in the age group of 0-4 years (n=15, 50%), followed by 4-8 years (n=12, 40%) and 8-12 years (n=3, 10%) (Table 10, Figure 1). ). Mann (2013) observed maximum renal failure cases in the age group of 4-8 years. Supriya (2019) observed mean age of dogs affected with renal failure as  $6.12 \pm 0.47$  years

**Table 10: Age wise and IRIS stage distribution of dogs presented with AKD as per IRIS staging of AKI (n=30).**

| Age group (years) | Acute kidney disease (Serum Cr: mg/dL) |                          |                          |                       | Overall (n=30)        |
|-------------------|----------------------------------------|--------------------------|--------------------------|-----------------------|-----------------------|
|                   | Stage 2 (1.7-2.5) (n=10)               | Stage 3 (2.6-5.0) (n=12) | Stage 4 (5.1-10.0) (n=7) | Stage 5 (>10.0) (n=1) |                       |
| <2                | 1                                      | 0                        | 1                        | 0                     | 2 (6.66) <sup>1</sup> |
| 2-4               | 2                                      | 5                        | 5                        | 1                     | 13 (43.33)            |
| 4-6               | 1                                      | 3                        | 1                        | 0                     | 5 (16.66)             |
| 6-8               | 3                                      | 4                        | 0                        | 0                     | 7 (23.33)             |
| 8-10              | 1                                      | 0                        | 0                        | 0                     | 1 (3.33)              |
| 10-12             | 2                                      | 0                        | 0                        | 0                     | 2 (6.66)              |
| <b>Total</b>      | <b>10 (33.3)</b>                       | <b>12 (40)</b>           | <b>7 (23.3)</b>          | <b>1 (3.3)</b>        | <b>30 (100)</b>       |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30

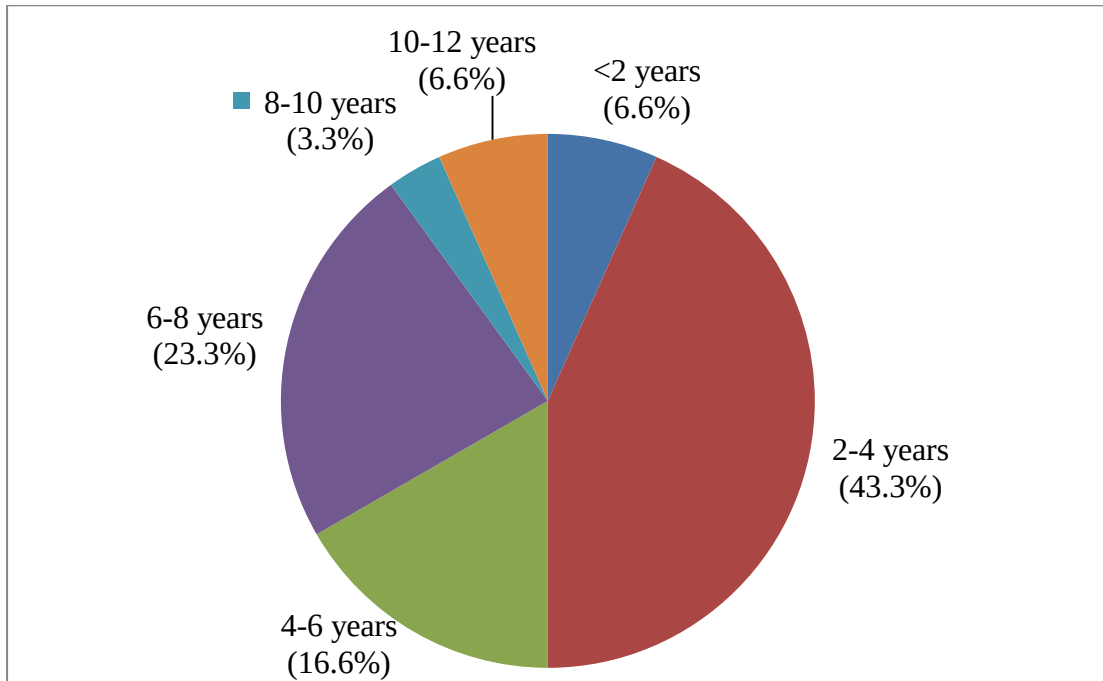


**Figure 1: Age wise distribution of dogs affected with acute kidney disease (n=30)**

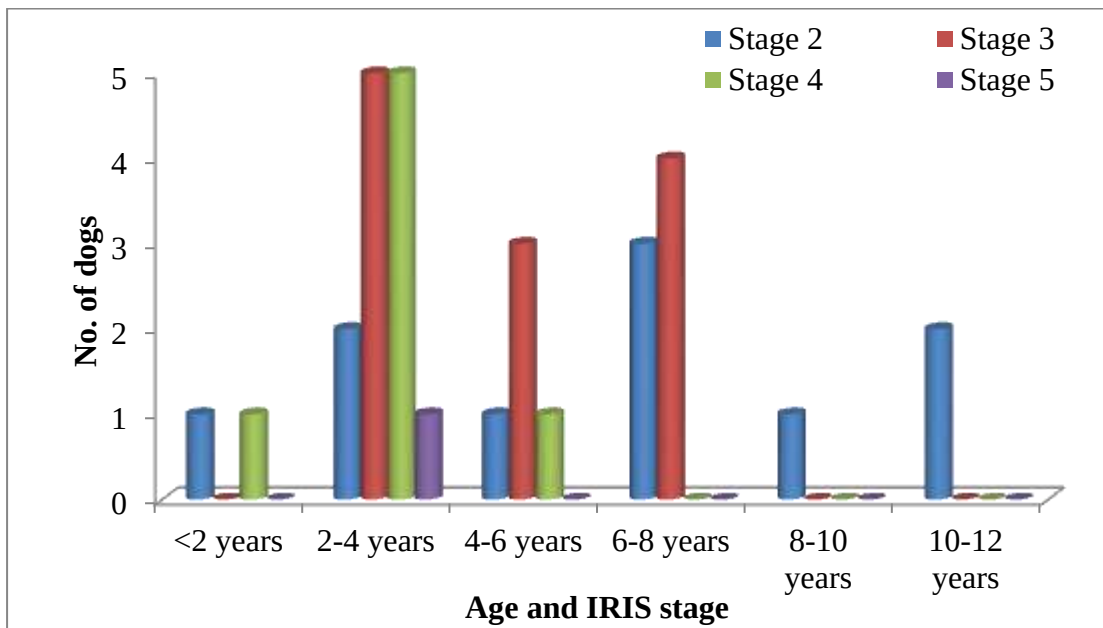


**Figure 2: Age wise and IRIS stage distribution of dogs affected with acute kidney disease (n=30)**

#### 4.1.2 Gender

AKD was observed more in male dogs (63.33%) than female dogs (37.66%) (Table 11). Eubig et al. (2005) also observed that intact male dogs were more affected than female dogs. The higher percentage of males may be due to the preference of owners to keep male dogs than female dog.

**Table 11: Gender and IRIS stage wise grouping of dogs presented with acute kidney disease (n=30)**

| Gender        | Acute kidney disease |                   |                  |                  | Overall<br>(n= 30)     |
|---------------|----------------------|-------------------|------------------|------------------|------------------------|
|               | Stage 2<br>(n=10)    | Stage 3<br>(n=12) | Stage 4<br>(n=7) | Stage 5<br>(n=1) |                        |
| <b>Male</b>   | 6 (20)               | 7 (23.33)         | 5 (16.66)        | 1 (3.33)         | 19 (63.3) <sup>1</sup> |
| <b>Female</b> | 4 (13.33)            | 5 (16.66)         | 2 (6.6)          | 0                | 11 (36.6)              |
| <b>Total</b>  | <b>10 (33.3)</b>     | <b>12 (40)</b>    | <b>7 (23.33)</b> | <b>1 (3.33)</b>  | <b>30 (100)</b>        |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30

#### 4.1.3 Breed

Among various canine breeds, Labrador retrievers were found to be more affected (36.6%) followed by Pug (10%) and Pitbull (10%) (Table 12, Figure 3). The higher prevalence among Labradors can be due to higher population of that particular breed in Punjab region.

**Table 12: Breed and IRIS stage wise distribution of dogs with acute kidney disease (n=30)**

| Breed                   | Acute kidney disease |                   |                  |                  | Overall<br>(n=30)      |
|-------------------------|----------------------|-------------------|------------------|------------------|------------------------|
|                         | Stage 2<br>(n=10)    | Stage 3<br>(n=12) | Stage 4<br>(n=7) | Stage 5<br>(n=1) |                        |
| <b>Labrador</b>         | 6                    | 3                 | 2                | 0                | 11 (36.6) <sup>1</sup> |
| <b>Pug</b>              | 1                    | 1                 | 1                | 0                | 3 (10)                 |
| <b>Pitbull</b>          | 1                    | 2                 | 0                | 0                | 3 (10)                 |
| <b>Beagle</b>           | 1                    | 1                 | 0                | 0                | 2 (6.6)                |
| <b>Non-descript</b>     | 0                    | 1                 | 1                | 0                | 2 (10)                 |
| <b>American Bully</b>   | 1                    | 1                 | 0                | 0                | 2 (6.6)                |
| <b>Mastiff</b>          | 0                    | 0                 | 0                | 1                | 1 (3.3)                |
| <b>Rottweiler</b>       | 0                    | 1                 | 0                | 0                | 1 (3.3)                |
| <b>Golden Retriever</b> | 0                    | 0                 | 1                | 0                | 1 (3.3)                |
| <b>Saint Bernard</b>    | 0                    | 1                 | 0                | 0                | 1 (3.3)                |
| <b>German shepherd</b>  | 0                    | 0                 | 1                | 0                | 1 (3.3)                |
| <b>Spitz</b>            | 0                    | 0                 | 1                | 0                | 1 (3.3)                |
| <b>Bull terrier</b>     | 0                    | 1                 | 0                | 0                | 1 (3.3)                |
| <b>Total</b>            | <b>10 (33.3)</b>     | <b>12 (40)</b>    | <b>7 (23.3)</b>  | <b>1 (3.3)</b>   | <b>30 (100)</b>        |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30

In a study conducted by Thade et al. (2019), highest prevalence was reported in Labrador (21.05%) followed by German Shephard (15.79%) and lowest (5.26%) in Boxer, Doberman, Pomeranian. Saravanan et al. (2012) also observed higher prevalence of renal failure among Labrador breeds. Dogra (2018) and Supriya (2019) reported Labrador retriever as the most presented breed for renal failure.

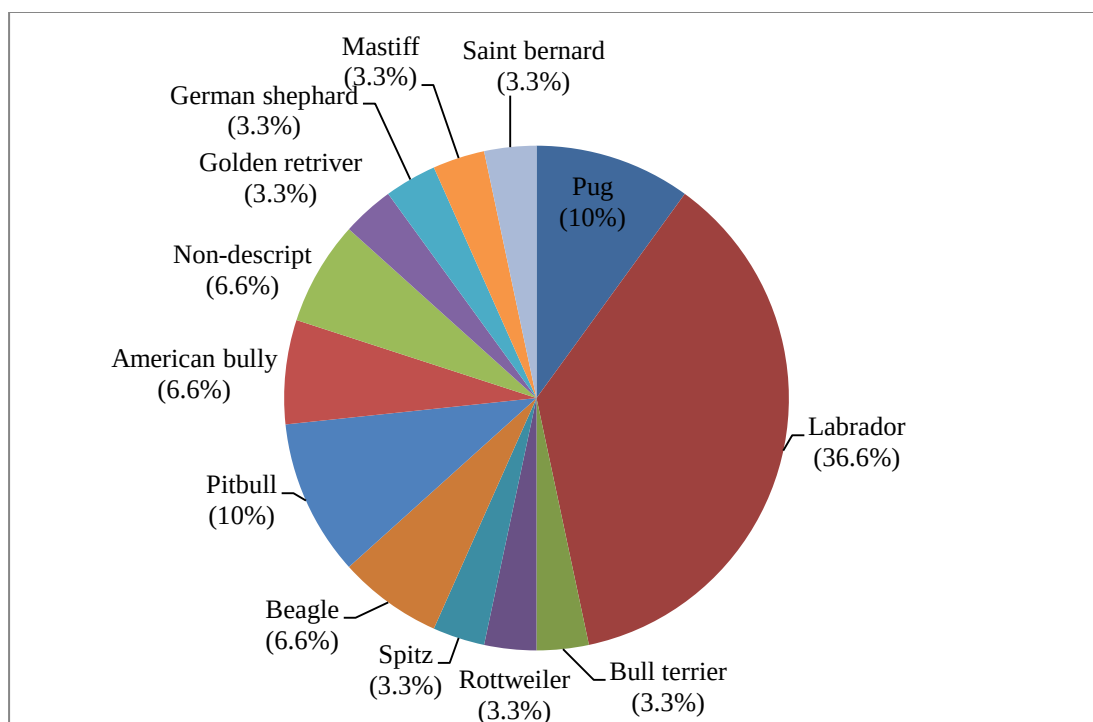


**Figure 3: Breed wise distribution of dogs affected with acute kidney disease (n=30)**

#### 4.2 History and Clinical signs

Major clinical entities among AKD dogs were inappetance (76.65%) (Table 13, Figure 4) followed by vomition (63.33%), lethargy (43.33%). Other signs included fever, retching, halitosis, oral ulceration, necrosis of tip of tongue, diarrhoea and melena. Somewhat similar signs were also observed by Chen et al. (2020) which included anorexia (84%), lethargy (77%), vomiting (55%) and diarrhoea (37%).

Tufani et al. (2015) observed vomition (75%), being the most common sign in dogs affected with AKD followed by anorexia (50%) and depression (37.5%). Reduced feed intake, vomiting, lethargy were associated with uraemia in dogs (Kingma et al., 2006). GI complications like vomiting, diarrhoea, inappetance were sometimes only or the very first sign observed by the owner (Braun & Lefebvre

2008). Possible causes for vomition in AKD can be due to effects of various uremic toxins on dopaminergic receptors in CTZ zone (Kumar, 2017). Uremic ulceration is an important factor for development of uremic breath, oral ulceration and melena (Lund et al., 1999).

**Table 13: Clinical signs in dogs presented with acute kidney disease (n=30)**

| History/signs              | Acute kidney disease |                   |                  |                  | Overall<br>(n=30)      |
|----------------------------|----------------------|-------------------|------------------|------------------|------------------------|
|                            | Stage 2<br>(n=10)    | Stage 3<br>(n=12) | Stage 4<br>(n=7) | Stage 5<br>(n=1) |                        |
| <b>Inappetance</b>         | 8                    | 9                 | 5                | 1                | 23 (76.6)              |
| <b>Vomiting</b>            | 6                    | 9                 | 4                | 0                | 19 (63.3) <sup>1</sup> |
| <b>Lethargic</b>           | 2                    | 6                 | 4                | 1                | 13 (43.3)              |
| <b>Tick infestation</b>    | 5                    | 3                 | 2                | 0                | 10 (33.3)              |
| <b>Melena</b>              | 2                    | 3                 | 2                | 0                | 7 (23.3)               |
| <b>Fever</b>               | 5                    | 1                 | 0                | 1                | 7 (23.3)               |
| <b>Diarrhea</b>            | 1                    | 3                 | 3                | 0                | 7 (23.3)               |
| <b>Retching</b>            | 3                    | 1                 | 3                | 0                | 7 (23.3)               |
| <b>Halitosis</b>           | 1                    | 1                 | 1                | 1                | 4 (13.3)               |
| <b>Pale mucus membrane</b> | 1                    | 2                 | 1                | 0                | 4 (13.3)               |
| <b>Polydipsia</b>          | 2                    | 1                 | 1                | 0                | 4 (13.3)               |
| <b>Hematuria</b>           | 2                    | 1                 | 0                | 0                | 3 (10)                 |
| <b>Epistaxis</b>           | 1                    | 2                 | 0                | 0                | 3 (10)                 |
| <b>Ascites</b>             | 0                    | 1                 | 1                | 0                | 3 (10)                 |
| <b>Anuria</b>              | 1                    | 0                 | 0                | 1                | 2 (6.6)                |
| <b>Hemoptysis</b>          | 2                    | 0                 | 0                | 0                | 2 (6.6)                |
| <b>Polyuria</b>            | 0                    | 1                 | 1                | 0                | 2 (6.6)                |
| <b>Oliguria</b>            | 0                    | 1                 | 1                | 0                | 2 (6.6)                |
| <b>Vaginal discharge</b>   | 1                    | 1                 | 0                | 0                | 2 (6.6)                |
| <b>Coughing</b>            | 0                    | 0                 | 2                | 0                | 2 (6.6)                |
| <b>Dental tartar</b>       | 0                    | 0                 | 1                | 0                | 1 (3.3)                |
| <b>Weight loss</b>         | 0                    | 1                 | 0                | 0                | 1 (3.3)                |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30



**Plate 5 (a): Petechial hemorrhages**



**Plate 5 (b): Hemoptysis**

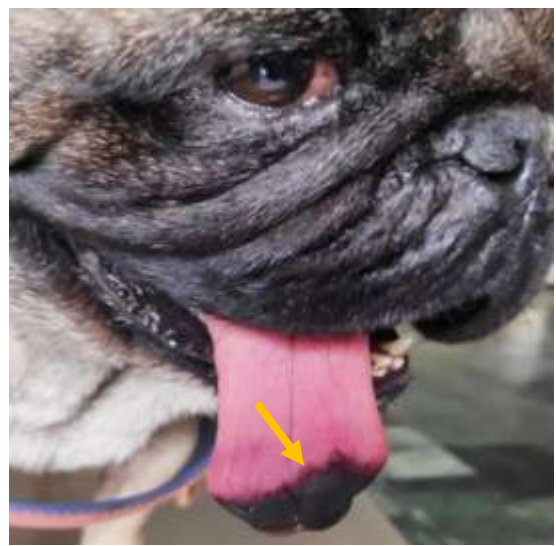
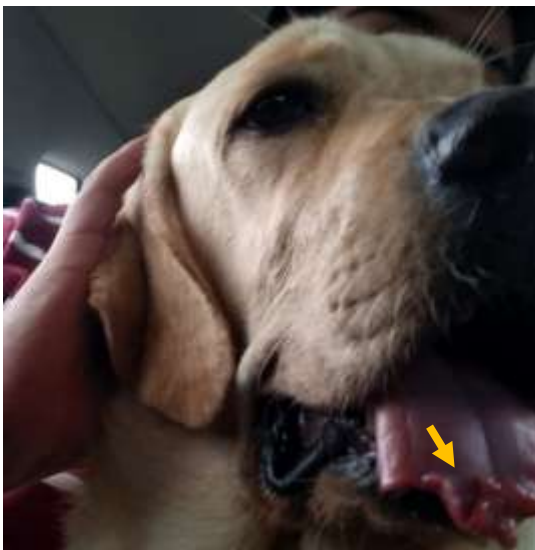


**Plate 5 (c, d): Necrosis of tip of tongue**

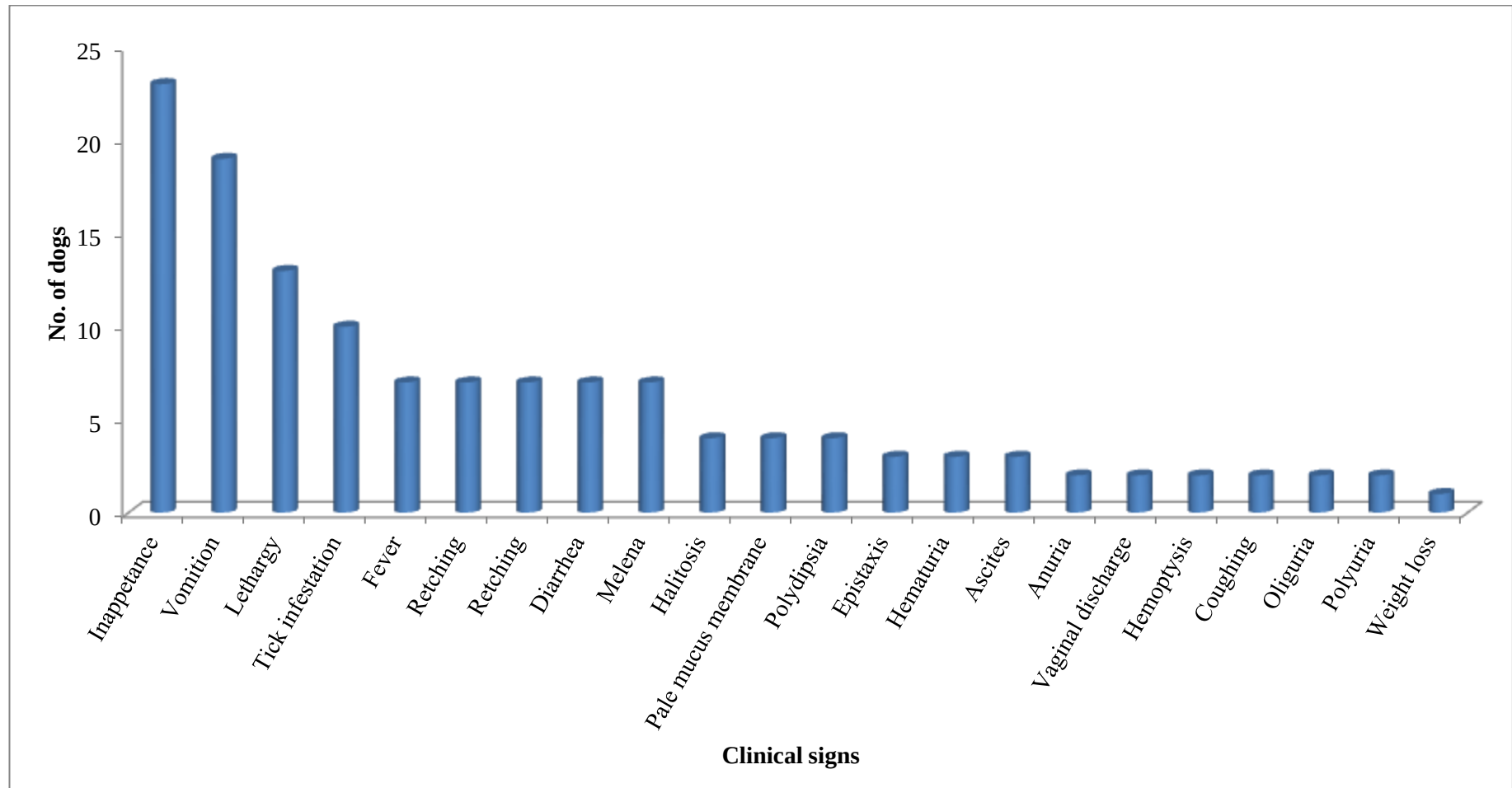


**Figure 4: Clinical signs in dogs affected with acute kidney disease (n=30)**

Oliguria was one of the most striking clinical finding observed in end stage renal disease which might be due to decreased renal blood flow, decreased GFR and subsequently decreased urine output (Hesse & Neiger, 2009).

### 4.3 Physical examination

#### 4.3.1 Rectal temperature

The mean rectal temperature ( $101.79 \pm 0.29$  °F) did not vary significantly from control group. Mazzaferro et al. (2004) observed mean temperature around 101°F in 4 dogs with AKD due to grape intoxication.

#### 4.3.2 Heart rate

Heart rate in AKD dogs was significantly lower  $121.16 \pm 4.33$ /min than control group (Table 14). Richards et al. (1983) also observed significantly lower heart rate in all AKD dogs as compared to the control group.

#### 4.3.3 Respiration rate

Respiration rate varied from 14-110/min yet mean respiration rate was not significantly different from the control group.

**Table 14: Vital body parameters in healthy (n=20) and acute kidney disease dogs (n=30)**

| Parameters                           | Reference range* | Healthy (n=20)                                                    | AKD (n=30)                             |
|--------------------------------------|------------------|-------------------------------------------------------------------|----------------------------------------|
| <b>Rectal temperature (°F)</b>       | 101-103          | $101.88 \pm 0.17^1$<br>(100-103) <sup>2</sup><br>102 <sup>3</sup> | $101.79 \pm 0.29$<br>(98-104.6)<br>102 |
| <b>Heart rate (per minute)</b>       | 70-120           | $132.40^a \pm 2.78$<br>(102-151)<br>134.5                         | $121.16^b \pm 4.33$<br>(80-166)<br>122 |
| <b>Respiratory rate (per minute)</b> | 18-34            | $32.40 \pm 1.24$<br>(20-41)<br>32.5                               | $36.63 \pm 3.15$<br>(14-110)<br>35     |

\*Ref: Textbook of Small Animal Practice Saunders 3rd edition (2006)

<sup>1</sup>Mean  $\pm$  SEM

<sup>2</sup>Figures in parentheses indicate range

<sup>3</sup>Median

Values in different columns with different alphabets differ significantly at  $P \leq 0.05$  as compared to control

Table 15: Vital body parameters in AKD dogs as per IRIS staging and UP:UC criteria

| Parameters                           | Acute kidney disease (Serum Cr: mg/dL) |                                   |                                   |                           | UP:UC staging                          |                                             |                                  | Overall (n=30)                   |
|--------------------------------------|----------------------------------------|-----------------------------------|-----------------------------------|---------------------------|----------------------------------------|---------------------------------------------|----------------------------------|----------------------------------|
|                                      | Stage 2<br>(1.7-2.5)<br>(n=10)         | Stage 3<br>(2.6-5.0)<br>(n=12)    | Stage 4<br>(5.1-10)<br>(n=7)      | Stage 5<br>(>10)<br>(n=1) | Non protein<br>uric<br>(<0.2)<br>(n=4) | Mild protein<br>uric<br>(0.2-0.5)<br>(n=13) | Proteinuric<br>(>0.5)<br>(n=13)  |                                  |
| <b>Rectal temperature (°F)</b>       | 101.31±0.72<br>(98-104.6)<br>101.45    | 102.07±0.32<br>(100-104)<br>102.2 | 101.82±0.48<br>(100.6-104)<br>101 | 103                       | 102.05±0.41<br>(101-103)<br>102.1      | 102.03±0.50<br>(98.4-104.6)<br>102.5        | 101.46±0.44<br>(98-104)<br>101.7 | 101.79±0.29<br>(98-104.6)<br>102 |
| <b>Heart rate (per minute)</b>       | 118.80±6.44<br>(80-144)<br>121         | 123.41±7.61<br>(88-166)<br>127.5  | 116.42±9.48<br>(96-162)<br>100    | 151                       | 123.00±10.93<br>(99-143)<br>125        | 126±7.61<br>(90-166)<br>122                 | 115.80±5.75<br>(80-144)<br>122   | 121.66±4.33<br>(80-166)<br>122   |
| <b>Respiratory rate (per minute)</b> | 37.00±3.59<br>(21-60)<br>38.5          | 31.66±3.40<br>(14-60)<br>30.5     | 44.00±11.20<br>(24-110)<br>32     | 41                        | 33.5±2.10<br>(29-38)<br>33.5           | 40.77±6.65<br>(14-110)<br>39                | 33.46±2.90<br>(22-60)<br>32      | 36.63±3.15<br>(14-110)<br>35     |

#### **4.4 Blood parameters of dogs affected with AKD**

##### **4.4.1 Haematological parameters in dogs affected with AKD**

Mean haemoglobin, PCV, TEC levels were significantly lower in AKD dogs than the control group (Table 16). Four dogs were severely anaemic which may be a pre renal factor for AKD (Claus et al., 2008). Haemolysis of red cells may be due to increased uremic toxins in blood and over expression of enzyme phosphatidyl serine on RBC membrane, which enhances the recognition of damaged red blood cells by macrophage system, leading to their subsequent destruction (Bonomini & Sirolli, 2003). Kumar et al. (2019) also reported that dogs suffering from acute renal failure had anaemia and reduced PCV with elevated TLC and neutrophil count.

Mean TLC, absolute neutrophil & lymphocyte counts and N:L were significantly higher than control group indicating that AKD may be associated with infection in dogs (Grauer, 1996). Overall Neutrophil to lymphocyte (N:L) ratio was significantly higher among all groups of renal failure as compared to the control group dogs (Table 16). Similar results were observed by Tufani et al. (2015) in an epidemiological study conducted in 9149 dogs.

##### **4.4.2 Serum biochemical parameters in dogs affected with acute kidney disease**

BUN and creatinine levels were significantly ( $P \leq 0.05$ ) higher and BUN/ Cr lower in all AKD dogs as compared to the control group (Table 18).

Jeong et al. (2006) concluded that BUN and serum creatinine are indicators for assessment of renal functioning and their elevations are observed in canine renal insufficiency.

Heiene et al. (2007) reported that serum urea and creatinine analysis has to be done in correlation with routine urine analysis and is considered as very initial step to evaluate dogs suspected for renal insufficiency. Normally creatinine is produced and released by skeletal muscles and thus served as an endogenous marker of GFR. Urea is an insensitive marker as its rate varies according to various conditions. Liver being actively involved in urea synthesis, its filtration by kidneys and reabsorption by renal tubules is a constant process. The rate of absorption depends on urine flow rate, GFR, thus it increases in dehydrated animals. Other conditions like fever, GIT bleeding and increased protein intake also increase BUN. Thus, plasma urea marker is not a good indicator of GFR.

**Table 16: Haematological profile in dogs with acute kidney disease (n=30) as per IRIS staging**

| Parameters                                            | Reference range* | Control (n=20)                                                                       | Acute kidney disease                                        |                                                     |                                                |               | Overall (n=30)                                          | < Ref value (n) | >Ref value (n) |
|-------------------------------------------------------|------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------|------------------------------------------------|---------------|---------------------------------------------------------|-----------------|----------------|
|                                                       |                  |                                                                                      | Stage 2 (n=10)                                              | Stage 3 (n=12)                                      | Stage 4 (n=7)                                  | Stage 5 (n=1) |                                                         |                 |                |
| <b>Hb (g/dL)</b>                                      | 12-18            | 15.10 <sup>A</sup> ±0.40 <sup>1</sup><br>(10.5-18) <sup>2</sup><br>15.4 <sup>3</sup> | 9.64 ±1.18<br>(3.9-14.2)<br>10.15                           | 10.65 ±1.37<br>(1.9-16.2)<br>12.6                   | 9.64 ±1.34<br>(2.9-12.5)<br>11.3               | 13            | 10.15 <sup>B</sup> ±0.73<br>(1.9-16.2)<br>11.65         | 15              | 0              |
| <b>TEC (×10<sup>6</sup> per mm<sup>3</sup>)</b>       | 5.5-8.5          | 6.13 <sup>A</sup> ±0.14<br>(4.44-7.04)<br>6.01                                       | 4.81 <sup>b</sup> ±0.51<br>(2.09-6.72)<br>5.36 <sup>3</sup> | 4.91 <sup>b</sup> ±0.54<br>(0.93-7.14)<br>5.73      | 4.29 <sup>b</sup> ±0.47<br>(1.88-5.41)<br>4.85 | 5.6           | 4.75 <sup>B</sup> ±0.29<br>(0.93-7.14)<br>5.32          | 16              | 0              |
| <b>PCV (%)</b>                                        | 37-55            | 45.45 <sup>A</sup> ±1.10<br>(33-51)<br>45.85                                         | 30.29 ±2.89<br>(13.9-42.4)<br>32.1                          | 32.34 ±4.08<br>(6.1-50)<br>37.8                     | 27.85 ±3.75<br>(8.9-36.8)<br>32                | 39            | 30.83 <sup>B</sup> ±2.06<br>(6.1-50)<br>32.9            | 19              | 0              |
| <b>Platelets (×10<sup>3</sup> per mm<sup>3</sup>)</b> | 200-800          | 293.15 <sup>A</sup> ±30.95<br>(71-492)<br>344                                        | 218.50±69.26<br>(18-729)<br>161                             | 218.41±32.83<br>(71-432)<br>217.5                   | 152.28±25.64<br>(84-258)<br>152                | 305           | 205.90 <sup>B</sup> ±27.01<br>(18-729)<br>184.5         | 15              | 0              |
| <b>TLC (cu mm)</b>                                    | 6000-17000       | 12385 <sup>A</sup> ±563.2<br>(8400-17200)<br>12300                                   | 24570±7763.74<br>(2400-90100)<br>18770                      | 16780±3356.98<br>(4460-45280)<br>12955 <sup>3</sup> | 15505.71±2379.65<br>(7200-22900)<br>16500      | 15500         | 19036.6 <sup>B</sup> ±2958.9<br>(2400-90100)<br>16200   | 2               | 13             |
| <b>Absolute Neutrophil count (per cu mm)</b>          | 3000-11500       | 8650.45 <sup>A</sup> ±383.3<br>2<br>(5880-12384)<br>8671.5                           | 21645.24±7062.68<br>(1296-81090)<br>17172.3                 | 11569.65±2215.15<br>(1811.2-24640)<br>9781.5        | 13304.62±2323.42<br>(4896-21062.4)<br>15510    | 13640         | 16760.4 <sup>B</sup> ±2725.2<br>(1296-81090)<br>14048.3 | 1               | 16             |
| <b>Absolute Lymphocyte count (per cu mm)</b>          | 1000-4800        | 3697.0 <sup>A</sup> ±221.8<br>(2436-6708)<br>3496                                    | 2918.2±773.11<br>(829.2-9010)<br>2064.7                     | 1540.33±237.4<br>(356.8-2702)<br>1769.6             | 2100.74 ±538.01<br>(606.6-4580)<br>2016        | 1860          | 2141.04 <sup>B</sup> ±310.45<br>(356.8-9010)<br>1835.6  | 8               | 0              |
| <b>N:L</b>                                            | 2.1-8.84         | 2.39 <sup>A</sup> ±0.08<br>(1.56-3.12)<br>2.37                                       | 8.13 ±2.05<br>(1.17-24)<br>5.82                             | 10.90 ±3.67<br>(1-49)<br>7.33                       | 10.04 ±3.18<br>(2.42-24)<br>6.14               | 7.33          | 10.41 <sup>B</sup> ±1.77<br>(1.17-49)<br>7.33           | 1               | 13             |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)<sup>1</sup> Mean± SEM<sup>2</sup> Figures in parentheses indicate range<sup>3</sup> Median

Values in different columns with different alphabets differ significantly at P≤0.05 as compared to control

Table 17: Haematological profiles in dogs with acute kidney disease (n=30) as per ACVIM and UP: UC staging

| Parameters                                        | Reference range* | Control (n=20)                                                                       | ACVIM staging                                |                                             |                                            | UP:UC staging                                   |                                                |                                                | Overall (n=30)                                            | <Ref value (n) | >Ref value (n) |
|---------------------------------------------------|------------------|--------------------------------------------------------------------------------------|----------------------------------------------|---------------------------------------------|--------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------------|-----------------------------------------------------------|----------------|----------------|
|                                                   |                  |                                                                                      | Normotensive (mm/Hg) (n=14)                  | Pre-hypertensive (mm/Hg) (n=11)             | Hypertensive (mm/Hg) (n=4)                 | Non-proteinuric (mg/dl) (n=4)                   | Mild proteinuric (mg/dl) (n=13)                | Proteinuric (mg/dl) (n=13)                     |                                                           |                |                |
| Hb (g/dL)                                         | 12-18            | 15.10 <sup>A</sup> ±0.40 <sup>1</sup><br>(10.5-18) <sup>2</sup><br>15.4 <sup>3</sup> | 11.39±1.03<br>(3.9-16.2)<br>12.4             | 8.80±1.17<br>(1.9-15.1)<br>8.8              | 9.05 ±2.35<br>(3.9-13.1)<br>9.6            | 10.07 <sup>b</sup> ±1.39<br>(6.2-12.2)<br>10.95 | 10.18 <sup>b</sup> ±1.14<br>(1.9-15.8)<br>11.3 | 10.15 <sup>b</sup> ±1.23<br>(2.9-16.2)<br>12.1 | 10.15 <sup>B</sup> ±0.73<br>(1.9-16.2)<br>11.65           | 15             | 0              |
| TEC (×10 <sup>6</sup> per mm <sup>3</sup> )       | 5.5-8.5          | 6.13 <sup>A</sup> ±0.14<br>(4.44-7.04)<br>6.01                                       | 5.14±0.38<br>(2.09-6.72)<br>5.73             | 4.38 ±0.52<br>(0.93-7.14)<br>4.85           | 4.2±0.94<br>(1.73-5.75)<br>4.66            | 4.72 <sup>b</sup> ±0.44<br>(3.72-5.72)<br>4.72  | 4.67 <sup>b</sup> ±0.46<br>(0.93-6.21)<br>5.23 | 4.85 <sup>b</sup> ±0.49<br>(1.88-7.14)<br>5.67 | 4.75 <sup>B</sup> ±0.29<br>(0.93-7.14)<br>5.32            | 16             | 0              |
| PCV (%)                                           | 37-55            | 45.45 <sup>A</sup> ±1.10<br>(33-51)<br>45.85                                         | 34.68±2.79<br>(13.9-50)<br>35.65             | 26.68 ±3.34<br>(6.1-41.9)<br>27.3           | 27.22 ±6.88<br>(12.4-39)<br>28.75          | 31.05 <sup>b</sup> ±4.32<br>(18.6-37)<br>34.3   | 30.35 <sup>b</sup> ±3.36<br>(6.1-49.5)<br>32   | 31.24 <sup>b</sup> ±3.31<br>(8.9-50)<br>33.4   | 30.83 <sup>B</sup> ±2.06<br>(6.1-50)<br>32.9              | 19             | 0              |
| Platelets (×10 <sup>3</sup> per mm <sup>3</sup> ) | 200-800          | 293.15 <sup>A</sup> ±30.95<br>(71-492)<br>344                                        | 184.78 ±34.88<br>(18-348)<br>186.5           | 211.90±54.14<br>(78-729)<br>162             | 287.25±63.84<br>(121-432)<br>298           | 262.5±155.63<br>(90-729)<br>115.5               | 193.46 ±32.08<br>(24-432)<br>162               | 200.92 ±32.88<br>(18-348)<br>225               | 205.90 <sup>B</sup> ±27.01<br>(18-729)<br>184.5           | 15             | 0              |
| TLC (cu mm)                                       | 6000-17000       | 12385.00 <sup>A</sup> ±563.20<br>(8400-17200)<br>12300                               | 22019.29±5681.38<br>(2400-90100)<br>17715    | 18674.55±3331.55<br>(4460-45280)<br>16810   | 13127.5±2164.82<br>(8300-17840)<br>13185   | 15612.5±3810.74<br>(4900-22900)<br>17325        | 16965.38±3098.42<br>(4460-45280)<br>15500      | 22161.53 ±6053.05<br>(2400-90100)<br>16500     | 19036.66 <sup>B</sup> ±2958.96<br>(2400-90100)<br>16200   | 2              | 13             |
| Absolute Neutrophil count (per cu mm)             | 3000-11500       | 8650.50 <sup>A</sup> ±383.33<br>(5880-12384)<br>8671.5                               | 19709.35 ±5166.31<br>(1296-81090)<br>14757.9 | 16156 ±3233.54<br>(4103.2-42563.2)<br>15510 | 11214±2035.42<br>(6308-15342.4)<br>11602.8 | 13107.8±3046.12<br>(4312-18320)<br>14899.5      | 14998.2±3013.4<br>(4103.2-42563.2)<br>12720    | 19646.6 ±5507.23<br>(1296-81090)<br>15510      | 16760.42 <sup>B</sup> ±2725.22<br>(1296-81090)<br>14048.3 | 1              | 16             |
| Absolute Lymphocyte count (per cu mm)             | 1000-4800        | 3697.00 <sup>A</sup> ±221.83<br>(2436-6708)<br>3496                                  | 2210.71±576.5<br>(480-9010)<br>1700          | 2276.29 ±423.75<br>(356.8-4723.4)<br>2016   | 1913.5±245.15<br>(1304.4-2497.6)<br>1926   | 2504.75±816.67<br>(588-4580)<br>2425.5          | 1737.3 ±225.12<br>(356.8-3180)<br>1811.2       | 2432.86±641.44<br>(480-9010)<br>1776           | 2141.04 <sup>B</sup> ±310.45<br>(356.8-9010)<br>1835.6    | 8              | 0              |
| N:L                                               | 2.1-8.84         | 2.39 <sup>A</sup> ±0.08<br>(1.56-3.12)<br>2.37                                       | 12.29±3.25<br>(1.17-49)<br>9                 | 9.91 ±2.43<br>(2.42-24)<br>6.14             | 5.99±0.98<br>(3.16-7.33)<br>6.73           | 5.90 ±0.69<br>(4-7.33)<br>6.14                  | 10.37 ±2.04<br>(2.42-24)<br>11                 | 11.84 ±3.54<br>(1.17-49)<br>7.33               | 10.41 <sup>B</sup> ±1.77<br>(1.17-49)<br>7.33             | 1              | 13             |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)<sup>1</sup>Mean± SEM<sup>2</sup>Figures in parentheses indicate range<sup>3</sup>Median

Values in different columns with different alphabets differ significantly at P≤0.05 as compared to control

**Table 18: Blood biochemical parameters in dogs with acute kidney disease (n=30) as per IRIS staging**

| Parameters                     | Reference range* | Control (n=20)                                                                 | Acute kidney disease                         |                                               |                                               |               | Overall (n=30)                                    | < Ref value (n) | >Ref value (n) |
|--------------------------------|------------------|--------------------------------------------------------------------------------|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------------|---------------------------------------------------|-----------------|----------------|
|                                |                  |                                                                                | Stage 2 (n=10)                               | Stage 3 (n=12)                                | Stage 4 (n=7)                                 | Stage 5 (n=1) |                                                   |                 |                |
| <b>BUN (mg/dL)</b>             | 8.8-25.9         | 19.1 <sup>A</sup> ±1.33 <sup>1</sup><br>(9-31) <sup>2</sup><br>19 <sup>3</sup> | 33.9 <sup>b</sup> ±6.83<br>(13-74)<br>24     | 46 <sup>b</sup> ±7.95<br>(21-106)<br>35       | 100.71 <sup>a</sup> ±28.9<br>(29-240)<br>67   | 140           | 57.86 <sup>B</sup> ±9.26<br>(13-240)<br>40        | 0               | 21             |
| <b>Cr (mg/dL)</b>              | 0.5-1.6          | 0.82 <sup>A</sup> ±0.07<br>(0.4-1.5)<br>0.75                                   | 2.05 <sup>c</sup> ±0.07<br>(1.7-2.4)<br>2.1  | 3.37 <sup>b</sup> ±0.21<br>(2.6-4.83)<br>3.15 | 6.48 <sup>a</sup> ±0.53<br>(5.4-9.5)<br>6     | 20            | 4.21 <sup>B</sup> ±0.64<br>(1.7-20)<br>2.95       | 0               | 30             |
| <b>BUN/Cr</b>                  | 17.6-28.3        | 26.01 <sup>A</sup> ±2.67<br>(7.69-62.5)<br>21.66                               | 16.37±3.18<br>(6.6-37)<br>12.44              | 14.30±2.88<br>(5.95-40.76)<br>10.55           | 15.44±4.57<br>(4.46-40)<br>11.35              | 7             | 15.01 <sup>B</sup> ±1.85<br>(4.46-40.76)<br>11.26 | 21              | 3              |
| <b>Ca<sup>++</sup> (mg/dL)</b> | 8.7-11.8         | 10.35±0.15<br>(7.9-11)<br>10.55                                                | 10.67±0.33<br>(8.8-12.1)<br>10.7             | 9.92±0.45<br>(6.9-12)<br>10.5                 | 9.73±0.52<br>(6.91-11)<br>10.1                | 9             | 10.09±0.25<br>(6.9-12.1)<br>10.35                 | 4               | 3              |
| <b>P<sub>i</sub> (mg/dL)</b>   | 2.9-6.2          | 3.99 <sup>A</sup> ±0.15<br>(2.5-5.5)<br>4                                      | 4.83 <sup>b</sup> ±0.54<br>(1.8-7.1)<br>4.75 | 5.7 <sup>b</sup> ±0.49<br>(3.4-8.4)<br>5.7    | 10.92 <sup>a</sup> ±2.05<br>(5.8-19.1)<br>8.5 | 20            | 7.10 <sup>B</sup> ±0.81<br>(1.8-20)<br>6.2        | 1               | 10             |
| <b>Ca/P</b>                    | 1.9-3            | 2.68 <sup>A</sup> ±0.12<br>(1.68-4.28)<br>2.62                                 | 2.57±0.42<br>(1.64-6.11)<br>2.13             | 1.90±0.19<br>(1.08-3.11)<br>1.82              | 1.07±0.18<br>(0.52-1.75)<br>0.98              | 0.45          | 1.88 <sup>B</sup> ±0.19<br>(0.45-6.11)<br>1.76    | 19              | 2              |
| <b>Cr/P</b>                    | 0.1-0.2          | 0.22 <sup>A</sup> ±0.02<br>(0.08-0.48)<br>0.17                                 | 0.48±0.06<br>(0.29-0.94)<br>0.42             | 0.66±0.09<br>(0.35-1.42)<br>0.53              | 0.70±0.10<br>(0.31-1.01)<br>0.73              | 1             | 0.62 <sup>B</sup> ±0.05<br>(0.29-1.42)<br>0.53    | 0               | 30             |
| <b>Na<sup>+</sup> (mEq/L)</b>  | 140-154          | 129.05 <sup>A</sup> ±0.66<br>(122-136)<br>129                                  | 133.5±3.11<br>(118-156)<br>131               | 134.5±1.81<br>(127-146)<br>132                | 138.85±1.71<br>(132-144)<br>141               | 131           | 135.1 <sup>B</sup> ±1.34<br>(118-156)<br>132.5    | 20              | 1              |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)<sup>1</sup>Mean± SEM<sup>2</sup>Figures in parentheses indicate range<sup>3</sup>Median

Values in different columns with different alphabets differ significantly at P≤0.05 as compared to control

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Table 18 cont....

| Parameters                        | Reference range* | Control (n=20)                               | Acute kidney disease                             |                                                 |                                                |               | Overall (n=30)                                 | < Ref value (n) | >Ref value (n) |
|-----------------------------------|------------------|----------------------------------------------|--------------------------------------------------|-------------------------------------------------|------------------------------------------------|---------------|------------------------------------------------|-----------------|----------------|
|                                   |                  |                                              | Stage 2 (n=10)                                   | Stage 3 (n=12)                                  | Stage 4 (n=7)                                  | Stage 5 (n=1) |                                                |                 |                |
| <b>K<sup>+</sup></b><br>(mEq/L)   | 4.37-5.35        | 4.99±0.27<br>(3.9-10)<br>4.8                 | 4.91±0.26<br>(3.3-6.7)<br>4.95                   | 5.37±0.37<br>(3.2-7.5)<br>4.9                   | 5.48±0.60<br>(3.8-8)<br>4.8                    | 6.9           | 5.29±0.22<br>(3.2-8)<br>4.95                   | 3               | 9              |
| <b>Na/K</b>                       | 28.8-32.1        | 26.85±0.94<br>(12.7-32.82)<br>27.39          | 27.77±1.29<br>(19.40-35.75)<br>27.15             | 26.27±1.98<br>(17.3-40.93)<br>26.87             | 27.21±2.91<br>(16.62-36.84)<br>27.5            | 18.98         | 26.81±1.10<br>(16.62-40.93)<br>27.04           | 20              | 4              |
| <b>Mg<sup>++</sup></b><br>(mg/dL) | 1.2-2.4          | 2.00±0.02<br>(1.8-2.3)<br>2                  | 2.04±0.08<br>(1.7-2.6)<br>2                      | 2.07±0.06<br>(1.8-2.6)<br>2                     | 2.12±0.15<br>(1.5-2.9)<br>2.2                  | 2             | 2.07±0.05<br>(1.5-2.9)<br>2                    | 0               | 3              |
| <b>Cl<sup>-</sup></b><br>(mEq/L)  | 105-117          | 89.55 <sup>A</sup> ±0.79<br>(84-95)<br>89    | 104 ±2.78<br>(93-118)<br>103.5                   | 104.33 ±2.02<br>(89-113)<br>105.5               | 107.28 ±2.64<br>(100-121)<br>106               | 88.5          | 104.38 <sup>B</sup> ±1.45<br>(88.5-121)<br>105 | 15              | 3              |
| <b>Glucose</b><br>(mg/dL)         | 80-120           | 98.95±2.96<br>(69-136)<br>98.5               | 95.6±5.06<br>(78-122)<br>92                      | 104.91±9.06<br>(67-181)<br>102.5                | 109.14±14.17<br>(78-192)<br>100                | 91            | 102.33±5.09<br>(67-192)<br>98                  | 4               | 5              |
| <b>TB</b><br>(mg/dL)              | 0.1-0.6          | 0.14±0.01<br>(0.1-0.2)<br>0.1                | 0.43±0.11<br>(0.1-1.1)<br>0.3                    | 0.23±0.01<br>(0.1-0.3)<br>0.2                   | 1.38±1.16<br>(0.1-.8.4)<br>0.3                 | 0.4           | 0.57±0.27<br>(0.1-8.4)<br>0.3                  | 0               | 3              |
| <b>ALT</b><br>(U/L)               | 8.2-57           | 46.2±1.66<br>(34-63)<br>45                   | 109±64.18<br>(15-674)<br>35.5                    | 35.58±8.50<br>(13-103)<br>25.5                  | 55.71±16.84<br>(32-156)<br>37                  | 60            | 65.56±22.04<br>(13-674)<br>56.5                | 0               | 5              |
| <b>AST</b><br>(U/L)               | 8.9-49           | 35.45 <sup>A</sup> ±1.19<br>(27-47)<br>34.5  | 84 ±19.50<br>(34-240)<br>67.5                    | 62.75 ±8.82<br>(37-141)<br>51.5                 | 159.14±98.55<br>(42-750)<br>67                 | 144           | 95.03 <sup>B</sup> ±23.90<br>(34-750)<br>61    | 0               | 19             |
| <b>AKP</b><br>(U/L)               | 10.6-101         | 103.2 <sup>A</sup> ±8.94<br>(38-188)<br>98.5 | 366.4 <sup>a</sup> ±107.14<br>(55-1103)<br>267.5 | 129.66 <sup>b</sup> ±19.16<br>(52-247)<br>103.5 | 180.57 <sup>ab</sup> ±30.03<br>(71-275)<br>183 | 90            | 222.46 <sup>B</sup> ±40.88<br>(52-1103)<br>172 | 0               | 20             |
| <b>TP</b><br>(g/dL)               | 5.5-7.5          | 6.75±0.16<br>(5.7-8.3)<br>6.6                | 5.74±0.49<br>(3.2-8.1)<br>6.1                    | 6.59±0.40<br>(4.6-9.4)<br>6.4                   | 6.45±0.73<br>(3.7-9.6)<br>6                    | 7.8           | 6.26±0.30<br>(3.2-9.6)<br>6.16                 | 9               | 7              |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006); <sup>1</sup>Mean± SEM; <sup>2</sup>Figures in parentheses indicate range; <sup>3</sup>Median

Values in different columns with different alphabets differ significantly at P≤0.05 as compared to control

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Table 18 cont....

| Parameters                 | Reference range* | Control (n=20)                                      | Acute kidney disease                           |                                                     |                                                 |               | Overall (n=30)                                      | < Ref value (n) | >Ref value (n) |
|----------------------------|------------------|-----------------------------------------------------|------------------------------------------------|-----------------------------------------------------|-------------------------------------------------|---------------|-----------------------------------------------------|-----------------|----------------|
|                            |                  |                                                     | Stage 2 (n=10)                                 | Stage 3 (n=12)                                      | Stage 4 (n=7)                                   | Stage 5 (n=1) |                                                     |                 |                |
| <b>ALB (g/dL)</b>          | 2.6-4            | 3.84 <sup>A</sup> ±0.03<br>(3.6-4.2)<br>3.9         | 2.61 ±0.24<br>(1.2-3.4)<br>2.8                 | 2.59±0.20<br>(1.7-4.2)<br>2.45                      | 2.81 ± 0.67<br>(1.7-6.6)<br>1.9                 | 3.3           | 2.67 <sup>B</sup> ±0.18<br>(1.2-6.6)<br>2.6         | 15              | 1              |
| <b>GLB (g/dL)</b>          | 2.7-4.4          | 2.91 <sup>A</sup> ±0.16<br>(1.6-4.4)<br>2.9         | 3.13 ±0.38<br>(1.2-5.2)<br>2.95                | 3.98 ±0.36<br>(2.5-6.4)<br>3.4                      | 3.64 ±0.76<br>(1.7-7.8)<br>3.4                  | 4.5           | 3.59 <sup>B</sup> ±0.26<br>(1.2-7.8)<br>3.3         | 7               | 6              |
| <b>ALB/GLB</b>             | 0.59-1.1         | 1.42 <sup>A</sup> ±0.09<br>(0.88-2.62)<br>1.25      | 0.98±0.21<br>(0.42-2.83)<br>0.84               | 0.70±0.07<br>(0.34-1)<br>0.64                       | 1.11±0.47<br>(0.23-3.88)<br>0.62                | 0.73          | 0.90 <sup>B</sup> ±0.13<br>(0.23-3.88)<br>0.75      | 7               | 2              |
| <b>TP/ALB</b>              | 1.87-2.11        | 1.75 <sup>A</sup> ±0.04<br>(1.38-2.12)<br>1.79      | 2.29 <sup>b</sup> ±0.17<br>(1.35-3.33)<br>2.18 | 2.63 <sup>ab</sup> ±0.19<br>(2-3.9)<br>2.55         | 2.72 <sup>ab</sup> ±0.49<br>(1.25-5.33)<br>2.60 | 2.36          | 2.50 <sup>B</sup> ±0.14<br>(1.25-5.33)<br>2.32      | 1               | 18             |
| <b>GGT (U/L)</b>           | 1.9-2.7          | 2.02 <sup>A</sup> ±0.03<br>(1.8-2.6)<br>2           | 5.00 <sup>a</sup> ±0.73<br>(2-9)<br>5          | 6.00 <sup>a</sup> ±0.69<br>(2-11)<br>5              | 12.42 <sup>a</sup> ±2.79<br>(5-23)<br>13        | 6             | 7.16 <sup>B</sup> ±0.89<br>(2-23)<br>5.5            | 0               | 26             |
| <b>Cholesterol (mg/dL)</b> | 135-270          | 165.8 <sup>A</sup> ±8.01<br>(122-269)<br>154.5      | 211.2 ±14.57<br>(166-300)<br>193.5             | 218.08±12.12<br>(167-301)<br>209.5                  | 223.28±19.04<br>(188-299)<br>189                | 200           | 216.4 <sup>B</sup> ±7.92<br>(166-301)<br>200        | 0               | 6              |
| <b>Fibrinogen (g/L)</b>    | 0.15-0.3         | 0.157 <sup>A</sup> ±0.009<br>(0.100-0.222)<br>0.162 | 0.24 <sup>b</sup> ±0.02<br>(0.15-0.39)<br>0.20 | 0.199 <sup>b</sup> ±0.011<br>(0.135-0.281)<br>0.195 | 2.11 <sup>a</sup> ±0.25<br>(1.04-3.05)<br>2.1   | 0.27          | 0.218 <sup>B</sup> ±0.012<br>(0.104-0.392)<br>0.201 | 2               | 4              |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)

<sup>1</sup>Mean± SEM

<sup>2</sup>Figures in parentheses indicate range

<sup>3</sup>Median

Values in different columns with different alphabets differ significantly at P≤0.05 as compared to control

**Table 19: Blood biochemical parameters in dogs with acute kidney disease (n=30) as per ACVIM and UP: UC staging**

| Parameters                     | Reference range* | Control (n=20)                                                                 | ACVIM staging                                |                                            |                                                | UP:UC staging                                  |                                   |                                  | Overall (n=30)                                    | < Ref value (n) | >Ref value (n) |
|--------------------------------|------------------|--------------------------------------------------------------------------------|----------------------------------------------|--------------------------------------------|------------------------------------------------|------------------------------------------------|-----------------------------------|----------------------------------|---------------------------------------------------|-----------------|----------------|
|                                |                  |                                                                                | Normotensive (n=15)                          | Pre-hypertensive (n=11)                    | Hypertensive (n=4)                             | Non-proteinuric (n=4)                          | Mild proteinuric (n=13)           | Proteinuric (n=13)               |                                                   |                 |                |
| <b>BUN (mg/dL)</b>             | 8.8-25.9         | 19.1 <sup>A</sup> ±1.33 <sup>1</sup><br>(9-31) <sup>2</sup><br>19 <sup>3</sup> | 48.71±17.02<br>(13-240)<br>24                | 62.54±9.58<br>(29-140)<br>63               | 80.5±26<br>(25-140)<br>79                      | 56 ±5.31<br>(44-67)<br>57                      | 68.8±13.5<br>(13-140)<br>47       | 47.4±16.6<br>(19-240)<br>25      | 57.86 <sup>B</sup> ±9.26<br>(13-240)<br>40        | 0               | 21             |
| <b>Cr (mg/dL)</b>              | 0.5-1.4          | 0.82 <sup>A</sup> ±0.07<br>(0.4-1.5)<br>0.75                                   | 3.10 <sup>b</sup> ±0.57<br>(1.7-9.5)<br>2.35 | 4.48 <sup>b</sup> ±0.50<br>(2-6.6)<br>4.83 | 7.67 <sup>b</sup> ±4.12<br>(2.6-20)<br>4.05    | 3.72 ±0.80<br>(2.2-5.9)<br>3.4                 | 5.28 ±1.37<br>(1.8-20)<br>3.3     | 3.29±0.44<br>(1.7-6.5)<br>2.7    | 4.21 <sup>B</sup> ±0.64<br>(1.7-20)<br>2.95       | 0               | 30             |
| <b>BUN/Cr</b>                  | 17.6-28.3        | 26.01 <sup>A</sup> ±2.67<br>(7.69-62.5)<br>21.66                               | 13.40 ±2.37<br>(6.28-40)<br>10.95            | 16.43 ±3.14<br>(4.46-37)<br>11.35          | 16.69±8.17<br>(5.95-40.76)<br>10.03            | 17.06 ±3.93<br>(11.35-28.63)<br>14.12          | 15.06 ±2.71<br>(6.6-40.7)<br>10.3 | 14.33±3.22<br>(4.46-40)<br>10.74 | 15.01 <sup>B</sup> ±1.85<br>(4.46-40.76)<br>11.26 | 21              | 3              |
| <b>Ca<sup>++</sup> (mg/dL)</b> | 8.7-11.8         | 10.35±0.15<br>(7.9-11)<br>10.5                                                 | 10.12±0.34<br>(6.9-11.7)<br>10.35            | 10.32±0.46<br>(6.91-12.1)<br>10.4          | 9.1±0.57<br>(7.8-10.6)<br>9                    | 10.5±0.68<br>(9-12.1)<br>10.5                  | 10.1±0.32<br>(7.8-12)<br>10.2     | 9.95±0.46<br>(6.9-12.1)<br>10.6  | 10.09±0.25<br>(6.9-12.1)<br>10.35                 | 4               | 3              |
| <b>P<sub>i</sub> (mg/dL)</b>   | 2.9-6.2          | 3.99 <sup>A</sup> ±0.15<br>(2.5-5.5)<br>4                                      | 6.2 ±1.20<br>(1.8-19.1)<br>5.15              | 7.62±1.02<br>(3.4-17)<br>7                 | 9.62±3.56<br>(4-20)<br>7.25                    | 6.3±0.92<br>(4-8.3)<br>6.45                    | 8.55±1.40<br>(3-20)<br>6.4        | 5.90±1.17<br>(1.8-19.1)<br>5.1   | 7.10 <sup>B</sup> ±0.81<br>(1.8-20)<br>6.2        | 1               | 10             |
| <b>Ca/P</b>                    | 1.9-3            | 2.68 <sup>A</sup> ±0.12<br>(1.68-4.28)<br>2.62                                 | 2.22±0.35<br>(0.52-6.11)<br>2.11             | 1.55±0.19<br>(0.6-3.11)<br>1.70            | 1.36 <sup>b</sup> ±0.46<br>(0.45-2.65)<br>1.17 | 1.81 <sup>b</sup> ±0.35<br>(1.08-2.77)<br>1.70 | 1.54±0.22<br>(0.45-3.3)<br>1.28   | 2.24±0.37<br>(0.52-6.11)<br>2.07 | 1.88 <sup>B</sup> ±0.19<br>(0.45-6.11)<br>1.76    | 19              | 2              |
| <b>Cr/P</b>                    | 0.1-0.2          | 0.22 <sup>A</sup> ±0.02<br>(0.08-0.48)<br>0.17                                 | 0.54±0.05<br>(0.31-0.94)<br>0.50             | 0.66±0.10<br>(0.29-1.42)<br>0.56           | 0.73±0.16<br>(0.41-1.05)<br>0.73               | 0.63±0.15<br>(0.30-1.01)<br>0.59               | 0.57±0.05<br>(0.35-1)<br>0.51     | 0.66±0.09<br>(0.29-1.42)<br>0.54 | 0.62 <sup>B</sup> ±0.05<br>(0.29-1.42)<br>0.53    | 0               | 30             |
| <b>Na<sup>+</sup> (mEq/L)</b>  | 140-154          | 129.05 <sup>A</sup> ±0.66<br>(122-136)<br>129                                  | 133.64±1.85<br>(118-146)<br>132.5            | 137.18 ±2.50<br>(129-156)<br>136           | 136.5±3.06<br>(131-144)<br>135.5               | 142 <sup>b</sup> ±6<br>(127-156)<br>142        | 133±1.9<br>(118-144)<br>132       | 135±1.6<br>(128-146)<br>132      | 135.1 <sup>B</sup> ±1.34<br>(118-156)<br>132.5    | 20              | 1              |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)<sup>1</sup>Mean± SEM<sup>2</sup>Figures in parentheses indicate range<sup>3</sup>Median

Values in different columns with different alphabets differ significantly at P≤0.05 as compared to control

cont....

Table 19 cont....

| Parameters                     | Reference range* | Control (n=20)                               | ACVIM staging                        |                                     |                                      | UP:UC staging                        |                                      |                                     | Overall (n=30)                                 | < Ref value (n) | >Ref value (n) |
|--------------------------------|------------------|----------------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|------------------------------------------------|-----------------|----------------|
|                                |                  |                                              | Normotensive (n=15)                  | Pre-hypertensive (n=11)             | Hypertensive (n=4)                   | Non-proteinuric (n=4)                | Mild proteinuric (n=13)              | Proteinuric (n=13)                  |                                                |                 |                |
| <b>K<sup>+</sup> (mEq/L)</b>   | 4.37-5.35        | 4.99±0.27<br>(3.9-10)<br>4.8                 | 5.38±0.35<br>(3.3-8)<br>4.95         | 5.07±0.39<br>(3.2-7.1)<br>4.8       | 5.8±0.45<br>(5-6.9)<br>5.65          | 5.1±0.38<br>(4.5-6.2)<br>4.85        | 5.13±0.37<br>(3.3-8)<br>4.8          | 5.52±0.36<br>(3.2-7.5)<br>5         | 5.29±0.22<br>(3.2-8)<br>4.95                   | 3               | 9              |
| <b>Na/K</b>                    | 28.8-32.1        | 26.85±0.94<br>(12.7-32.8)<br>27.39           | 26.07±1.49<br>(16.62-35.75)<br>27.04 | 28.64±2.18<br>(19.40-40.93)<br>27.5 | 24.01±2.06<br>(18.98-28.23)<br>24.40 | 28.13±2.01<br>(22.41-31.30)<br>29.40 | 27.45±1.74<br>(16.62-36.84)<br>27.08 | 25.77±1.79<br>(17.33-40.93)<br>26.4 | 26.81±1.10<br>(16.62-40.93)<br>27.04           | 20              | 4              |
| <b>Mg<sup>++</sup> (mg/dL)</b> | 1.2-2.4          | 2.00±0.02<br>(1.8-2.3)<br>2                  | 2.1±0.08<br>(1.8-2.9)<br>2           | 1.98±0.06<br>(1.5-2.2)<br>2         | 2.17±0.14<br>(2-2.6)<br>2.05         | 1.87±0.17<br>(1.5-2.3)<br>1.85       | 2.12±0.07<br>(1.9-2.9)<br>2.1        | 2.08±0.07<br>(1.8-2.6)<br>2         | 2.07±0.05<br>(1.5-2.9)<br>2                    | 0               | 3              |
| <b>Cl<sup>-</sup> (mEq/L)</b>  | 105-117          | 89.55 <sup>A</sup> ±0.79<br>(84-95)<br>89    | 104.85±1.98<br>(93-118)<br>105       | 105.5±2.27<br>(95-121)<br>103       | 98.37±5.57<br>(88.5-109)<br>98       | 99.8±4.68<br>(89-109)<br>101         | 104±2.32<br>(88.5-121)<br>103        | 106±1.97<br>(93-118)<br>107         | 104.38 <sup>B</sup> ±1.45<br>(88.5-121)<br>105 | 15              | 3              |
| <b>Glucose (mg/dL)</b>         | 80-120           | 98.95±2.96<br>(69-136)<br>98.5               | 95.92±8.20<br>(67-192)<br>90         | 104.27±5.51<br>(78-136)<br>100      | 99.75±3.37<br>(91-106)<br>101        | 117.75±21.12<br>(93-181)<br>98.5     | 96.53±4.74<br>(78-136)<br>91         | 103.38±8.88<br>(67-192)<br>100      | 102.33±5.09<br>(67-192)<br>98                  | 4               | 5              |
| <b>TB (mg/dL)</b>              | 0.1-0.6          | 0.14±0.01<br>(0.1-0.2)<br>0.1                | 1±0.6<br>(0.1-8.4)<br>0.3            | 0.2±0.02<br>(0.1-0.3)<br>0.3        | 0.27±0.04<br>(0.2-0.4)<br>0.25       | 0.23±0.03<br>(0.2-0.3)<br>0.2        | 0.88±0.63<br>(0.1-8.4)<br>0.3        | 0.37±0.09<br>(0.1-1.1)<br>0.2       | 0.57±0.27<br>(0.1-8.4)<br>0.3                  | 0               | 3              |
| <b>ALT (U/L)</b>               | 8.2-57           | 46.2±1.66<br>(34-63)<br>45                   | 86±46<br>(15-674)<br>30              | 54±14<br>(13-163)<br>64             | 38±8.3<br>(20-60)<br>36              | 32±6.1<br>(20-47)<br>30              | 49±12<br>(13-156)<br>36              | 93±50<br>(15-674)<br>38             | 65.56±22.04<br>(13-674)<br>56.5                | 0               | 5              |
| <b>AST (U/L)</b>               | 8.9-49           | 35.45 <sup>A</sup> ±1.19<br>(27-47)<br>34.5  | 123±50<br>(34-750)<br>58             | 63±5<br>(43-101)<br>62              | 100±26<br>(37-144)<br>110            | 65±8.3<br>(42-78)<br>69              | 132±53<br>(34-750)<br>67             | 68±15<br>(37-240)<br>49             | 95.03 <sup>B</sup> ±23.90<br>(34-750)<br>61    | 0               | 19             |
| <b>AKP (U/L)</b>               | 10.6-101         | 103.8 <sup>A</sup> ±9.23<br>(38-200)<br>98.5 | 303±81.1<br>(55-1103)<br>222         | 153±25.7<br>(52-275)<br>113         | 164±27.6<br>(86-213)<br>179          | 213±41.04<br>(97-275)<br>240         | 199±55.3<br>(52-808)<br>177          | 249±77.7<br>(55-1103)<br>154        | 222.46 <sup>B</sup> ±40.88<br>(52-1103)<br>172 | 0               | 20             |
| <b>TP (g/dL)</b>               | 5.5-7.5          | 6.75±0.16<br>(5.7-8.3)<br>6.6                | 5.92±0.39<br>(3.2-8.3)<br>6.1        | 6.21±0.56<br>(3.5-9.6)<br>6.2       | 5.72±0.53<br>(4.2-6.6)<br>6.05       | 5.7±0.9<br>(3.7-7.8)<br>5.65         | 6.09±0.43<br>(3.5-9.6)<br>6.1        | 6.60±0.47<br>(3.2-9.4)<br>6.6       | 6.26±0.30<br>(3.2-9.6)<br>6.16                 | 9               | 7              |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006); <sup>1</sup>Mean± SEM ; <sup>2</sup>Figures in parentheses indicate range; <sup>3</sup>Median  
Values in different rows with different alphabets differ significantly at P≤0.05 as compared to control

cont....

Table 19 cont....

| Parameters                 | Reference range* | Control (n=20)                                 | ACVIM staging                    |                                  |                                   | UP:UC staging                    |                                  |                                  | Overall (n=30)                                 | < Ref value (n) | >Ref value (n) |
|----------------------------|------------------|------------------------------------------------|----------------------------------|----------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|------------------------------------------------|-----------------|----------------|
|                            |                  |                                                | Normotensive (n=15)              | Pre-hypertensive (n=11)          | Hypertensive (n=4)                | Non-proteinuric (n=4)            | Mild proteinuric (n=13)          | Proteinuric (n=13)               |                                                |                 |                |
| <b>ALB (g/dL)</b>          | 2.6-4            | 3.84 <sup>A</sup> ±0.03<br>(3.6-4.2)<br>3.9    | 2.84±0.34<br>(1.2-6.6)<br>2.9    | 2.51±0.26<br>(1.7-4.2)<br>2.3    | 2.58±0.34<br>(2-3.3)<br>2.5       | 2.4±0.37<br>(1.7-3.4)<br>2.25    | 2.35±0.16<br>(1.7-3.5)<br>2.1    | 3.07±0.36<br>(1.2-6.6)<br>3      | 2.67 <sup>B</sup> ±0.18<br>(1.2-6.6)<br>2.6    | 15              | 1              |
| <b>GLB (g/dL)</b>          | 2.7-4.4          | 2.91 <sup>A</sup> ±0.16<br>(1.6-4.4)<br>2.9    | 3.07±0.24<br>(1.7-5.2)<br>3.05   | 3.71±0.54<br>(1.2-7.8)<br>3.8    | 4.95±0.73<br>(3.1-6.4)<br>5.15    | 3.30±1.04<br>(1.2-5.8)<br>3.1    | 3.73±0.42<br>(1.8-7.8)<br>3.4    | 3.53±0.35<br>(1.7-6.4)<br>3.3    | 3.59 <sup>B</sup> ±0.26<br>(1.2-7.8)<br>3.3    | 7               | 6              |
| <b>ALB/GLB</b>             | 0.59-1.1         | 1.42 <sup>A</sup> ±0.09<br>(0.88-2.62)<br>1.22 | 1.04±0.22<br>(0.42-3.88)<br>0.92 | 0.89±0.21<br>(0.23-2.83)<br>0.64 | 0.54±0.08<br>(0.34-0.73)<br>0.55  | 1.15±0.56<br>(0.34-2.83)<br>0.72 | 0.71±0.07<br>(0.23-1.20)<br>0.64 | 1.02±0.24<br>(0.42-3.88)<br>0.93 | 0.90 <sup>B</sup> ±0.13<br>(0.23-3.88)<br>0.75 | 7               | 2              |
| <b>TP/ALB</b>              | 1.87-2.11        | 1.75 <sup>A</sup> ±0.04<br>(1.38-2.12)<br>1.79 | 2.24±0.13<br>(1.25-3.33)<br>2.07 | 2.65±0.32<br>(1.35-5.33)<br>2.55 | 2.98±0.34<br>(2.36-3.9)<br>2.84   | 2.52±0.53<br>(1.35-3.9)<br>2.42  | 2.69±0.25<br>(1.82-5.33)<br>2.55 | 2.31±0.15<br>(1.25-3.33)<br>2.06 | 2.50 <sup>B</sup> ±0.14<br>(1.25-5.33)<br>2.32 | 1               | 18             |
| <b>GGT (U/L)</b>           | 1.9-2.7          | 2.02 <sup>A</sup> ±0.03<br>(1.8-2.6)<br>2      | 7.3±1.7<br>(2-23)<br>5           | 6.5±1.2<br>(2-15)<br>5           | 8±1.1<br>(6-11)<br>7.5            | 5.75±1.49<br>(2-9)<br>6          | 7.38±1.48<br>(2-20)<br>5         | 7.38±1.42<br>(3-23)<br>6         | 7.16 <sup>B</sup> ±0.89<br>(2-23)<br>5.5       | 0               | 26             |
| <b>Cholesterol (mg/dL)</b> | 135-270          | 165.8 <sup>A</sup> ±8.01<br>(122-269)<br>154.5 | 215.7±10.62<br>(166-300)<br>211  | 219.09±14.49<br>(182-299)<br>189 | 190.25±9.19<br>(167-209)<br>192.5 | 242±33.6<br>(182-301)<br>242     | 218±10.9<br>(167-291)<br>209     | 207±11<br>(166-300)<br>198       | 216.4 <sup>B</sup> ±7.92<br>(166-300)<br>200   | 0               | 6              |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)

<sup>1</sup>Mean± SEM

<sup>2</sup>Figures in parentheses indicate range

<sup>3</sup>Median

Values in different rows with different alphabets differ significantly at P≤0.05 as compared to control

The mean phosphorus levels,  $\text{Cr/P}_i$  were significantly higher and  $\text{Ca/P}_i$  significantly lower in AKD dogs (Table 18). Ritz and Schwenger (2005) stated that lack of phosphorus excretion and lack of active form of vitamin D fastens the progression of kidney dysfunction. Labato (2001) observed increased levels of phosphorus in AKD and stated that phosphorus retention in ARF signifies reduced GFR rather than course of disease. Giovaninni et al. (2013) reported that phosphorus retention occurs mainly due to decreased GFR values.

Significantly lower mean value of  $\text{Ca/P}$  ratio in present study was due to hyperphosphatemia with normal calcium levels. Serum calcium levels can be normal or slight reduced with increased phosphorus levels in AKD (Ross, 2011). Serum Sodium can be normal or slight elevated in AKD. Elevation of mean sodium i.e.;  $104.38 \pm 1.45$  was seen in all groups and differs significantly from healthy ones. Similar results were found by (Suetrong et al., 2016) in a study over 240 patients with concurrent sepsis. Hyperchloraemia could be due to renal vasoconstriction, decreased renal blood flow and thus glomerular filtration rate, chloride-induced thromboxane and increased response to renal vasoconstrictors (Suetrong et al., 2016).

There was no significant change in  $\text{K}^+$ , Mg, glucose, ALT and TP in AKD dogs. AST, ALKP, GGT, cholesterol and fibrinogen levels were significantly higher in AKD dogs while albumin was significantly lower than control dogs (Table 18).

Elevated levels of serum GGT in dogs affected with AKD in present study may be due to hepatic involvement or cholestasis. GGT levels are sensitive marker of cholestasis and are a valuable tool in early diagnosis of hepatic dysfunction (Katoch et al., 2017).

Infections, malabsorption due gastroenteritis, gastrointestinal bleeding and renal protein loss may be reason for hypoalbuminemia (Kovarikova, 2015).

Expansion in serum ALKP is more normal than expansion in serum ALT in AKD (Metcalf et al., 2002). This may be a reason for higher ALKP levels. Azotemia along with elevated liver enzymes may be indicate leptospirosis in dogs (Giovaninni et al., 2013).

Mastorilli et al. (2007) also reported elevation in GGT, ALP and AST in a retrospective study of 20 dogs affected with AKD and concluded that this can be due to circulatory hypoxia, metabolic acidosis, toxemia or cholestasis.

#### 4.4.3 Renal specific biomarkers in dogs affected with AKD

##### 4.4.3.1 Serum renal biomarkers

Biomarkers are considered to be measurable indicators for normal or abnormal metabolic changes (Frangogiannis, 2012). Commonly used biomarkers for renal insufficiency are SDMA, NGAL, Cystatin-C, RBP, Ig-G, UPC, etc., and are considered to be one of the efficient tools for early diagnosis of renal insult.

In present study, the mean levels of serum SDMA, NGAL and Cystatin C were significantly higher as compared to the control group (Table 21). Pearson correlation also showed moderate correlation of SDMA with serum creatinine values (Table 20).

**Table 20: Pearson's correlation test of serum creatinine in relation to various haematobiochemical, serum and urine biomarkers**

| Serum creatinine | Parameters                | r value             | Positive/negative |
|------------------|---------------------------|---------------------|-------------------|
|                  | Hb                        | 0.291 <sup>1</sup>  | +                 |
|                  | TEC                       | 0.097               | +                 |
|                  | PCV                       | 0.406               | +                 |
|                  | TLC                       | 0.237               | -                 |
|                  | Absolute Neutrophil count | 0.259               | -                 |
|                  | Absolute Lymphocyte count | 0.041               | -                 |
|                  | Platelets                 | 0.108               | -                 |
|                  | BUN                       | 0.544* <sup>1</sup> | +                 |
|                  | GGT                       | 0.439 <sup>1</sup>  | +                 |
|                  | Calcium                   | 0.220               | -                 |
|                  | Phosphorus                | 0.537* <sup>1</sup> | +                 |
|                  | Albumin                   | 0.477* <sup>1</sup> | +                 |
|                  | Cr/P                      | 0.444* <sup>1</sup> | +                 |
|                  | Serum SDMA                | 0.479* <sup>1</sup> | +                 |
|                  | Serum NGAL                | 0.317               | +                 |
|                  | Serum cystatin C          | 0.171               | -                 |
|                  | Serum cT I                | 0.149               | +                 |
|                  | Urine protein             | 0.249*              | +                 |
|                  | Urine UP:UC               | 0.128               | +                 |

<sup>1</sup>r values are significant at P<0.05

\* Moderate correlation

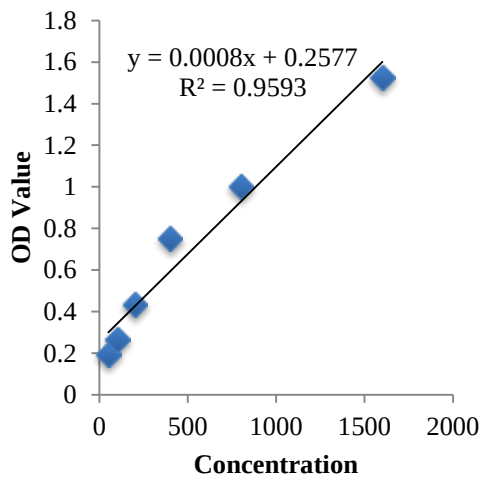


**Figure 5 (a): Sensitivity of serum SDMA in ELISA**

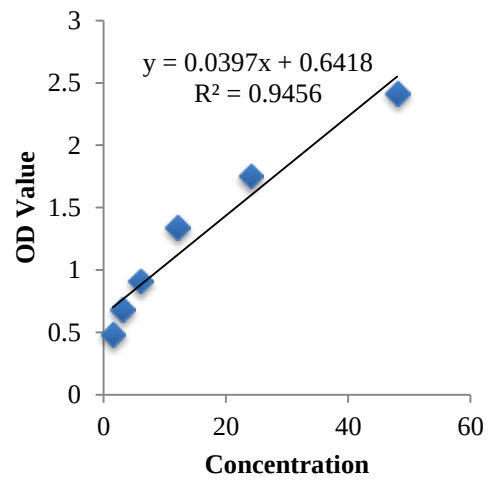


**Figure 5 (b): Sensitivity of serum NGAL in ELISA**

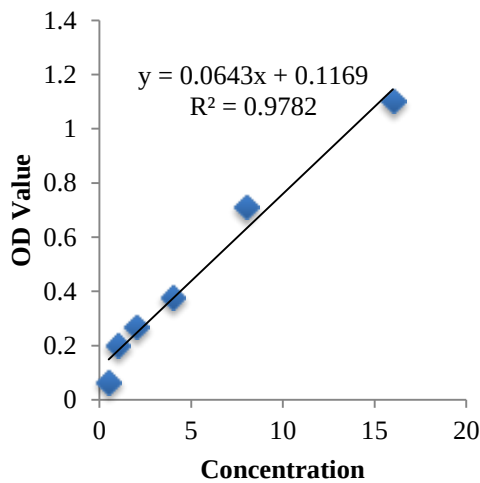


**Figure 5 (c): Sensitivity of serum cystatin C in ELISA**

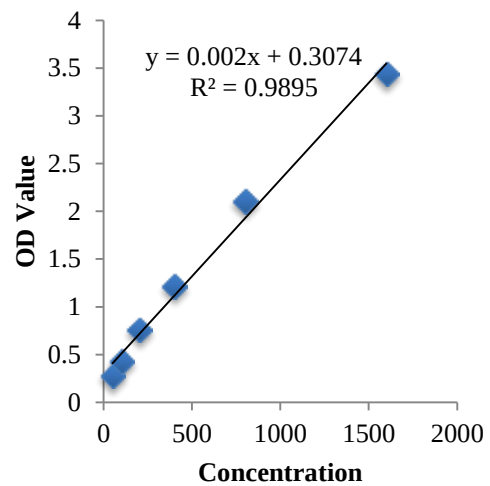


**Figure 5 (d): Sensitivity of serum cTn-1 in ELISA**

Nejat et al. (2012) in a prospective study also found higher levels of Cystatin C in AKI group as compared to the healthy. Trof et al. (2006) reported serum cystatin C concentration to be better than serum creatinine to detect acute changes of glomerular filtration. Sasaki et al. (2014) also reported higher levels of NGAL, Cystatin C and SCr in dogs affected with acute renal insult and concluded these markers as important tool for early diagnosis of renal insult.

Table 21: Serum renal biomarkers in dogs with acute kidney disease (n=20) as per IRIS staging

| Parameters               | Reference range* | Control (n=20)                                                                         | Acute kidney disease                               |                                                     |                                           | Overall (n=20)                                     | < Ref value (n) | >Ref value (n) |
|--------------------------|------------------|----------------------------------------------------------------------------------------|----------------------------------------------------|-----------------------------------------------------|-------------------------------------------|----------------------------------------------------|-----------------|----------------|
|                          |                  |                                                                                        | Stage 2 (n=10)                                     | Stage 3 (n=7)                                       | Stage 4 (n=3)                             |                                                    |                 |                |
| <b>SDMA (µg/dL)</b>      | 0-14             | 5.12 <sup>A</sup> ±5.17 <sup>1</sup><br>(0.37-11.62) <sup>2</sup><br>5.37 <sup>3</sup> | 43.62 ±1.80<br>(37.87-55.37)<br>41.62              | 48.94 ±1.66<br>(41.62-54.12)<br>49.12               | 72.04 ±10.11<br>(57.87-91.62)<br>66.62    | 49.74 <sup>B</sup> ±2.25<br>(37.87-91.62)<br>47.25 | 0               | 19             |
| <b>NGAL (ng/mL)</b>      | 12-13            | 7.06 <sup>A</sup> ±6.82<br>(0.18-15.57)<br>6.25                                        | 40.10 <sup>b</sup> ±1.31<br>(30.38-45.49)<br>40.76 | 19.32 <sup>a</sup> ±0.22<br>(18.29-19.87)<br>19.42  | 18.35 ±0.23<br>(18.04-18.82)<br>18.19     | 30.30 <sup>B</sup> ±1.98<br>(18.04-45.49)<br>29.29 | 0               | 20             |
| <b>Cystatin C (mg/L)</b> | 0-1.4            | 0.170 <sup>A</sup> ±0.161<br>(0.0001-0.6891)<br>0.091                                  | 1.02 <sup>b</sup> ±0.01<br>(0.94-1.07)<br>1.02     | 0.905 <sup>a</sup> ±0.008<br>(0.874-0.924)<br>0.917 | 0.63±0.06<br>(0.49-0.71)<br>0.68          | 0.91 <sup>B</sup> ±0.02<br>(0.49-1.07)<br>0.93     | 0               | 0              |
| <b>cT I (ng/dL)</b>      | 0-0.2            | 0.023±0.021<br>(0.006-0.092)<br>0.015                                                  | 0.016±0.002<br>(0.006-0.026)<br>0.017              | 0.012±0.002<br>(0.006-0.022)<br>0.011               | 0.026±0.0205<br>(0.0001-0.0668)<br>0.0117 | 0.0151±0.0026<br>(0.0001-0.0668)<br>0.0117         | 0               | 0              |

\*Ref: IDEXX (serum biomarkers), Saini (2014) (cT 1)

<sup>1</sup>Mean± SEM<sup>2</sup>Figures in parentheses indicate range<sup>3</sup>MedianValues in different rows with different alphabets differ significantly at  $P \leq 0.05$  as compared to control

**Table 22: Serum renal biomarkers in dogs with acute kidney disease (n=20) as per ACVIM and UP: UC staging**

| Parameters               | Reference range* | Control (n=20)                                                                         | ACVIM staging                                      |                                                    |                    | UP:UC staging         |                                       |                                        | Overall (n=20)                                     | < Ref value (n) | >Ref value (n) |
|--------------------------|------------------|----------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------------------------|--------------------|-----------------------|---------------------------------------|----------------------------------------|----------------------------------------------------|-----------------|----------------|
|                          |                  |                                                                                        | Normotensive (n=13)                                | Pre-hypertensive (n=6)                             | Hypertensive (n=1) | Non-proteinuric (n=2) | Mild proteinuric (n=6)                | Proteinuric (n=12)                     |                                                    |                 |                |
| <b>SDMA (µg/dL)</b>      | 0-14             | 5.12 <sup>A</sup> ±5.17 <sup>1</sup><br>(0.37-11.62) <sup>2</sup><br>5.37 <sup>3</sup> | 46.23±2.33<br>(37.87-66.62)<br>42.8                | 57.45 ±5.16<br>(45.37-91.62)<br>52.25              | 49.12              | 47.87, 51.62          | 53.91±5.36<br>(39.12-91.62)<br>47.86  | 47.66±2.45<br>(37.87-66.62)<br>46      | 49.74 <sup>B</sup> ±2.25<br>(37.87-91.62)<br>47.25 | 0               | 19             |
| <b>NGAL (ng/mL)</b>      | 12-13            | 7.06 <sup>A</sup> ±6.82<br>(0.18-15.57)<br>6.25                                        | 34.18 <sup>a</sup> ±2.71<br>(18.04-45.49)<br>39.47 | 23.70 <sup>b</sup> ±2.83<br>(18.19-42.72)<br>19.74 | 19.42              | 19.60, 19.87          | 34.39 ±3.51<br>(18.19-45.49)<br>40.83 | 30.01±2.80<br>(18.04-42.29)<br>29.29   | 30.30 <sup>B</sup> ±1.98<br>(18.04-45.49)<br>29.29 | 0               | 20             |
| <b>Cystatin C (mg/L)</b> | 0-1.4            | 0.170 <sup>A</sup> ±0.161<br>(0.0001-0.6891)<br>0.091                                  | 0.93±0.04<br>(0.49-1.07)<br>1.01                   | 0.87±0.02<br>(0.71-0.97)<br>0.89                   | 0.917              | 0.88, 0.87            | 0.93±0.03<br>(0.71-1.02)<br>0.96      | 0.91 ±0.04<br>(0.49-1.07)<br>0.93      | 0.91 <sup>B</sup> ±0.02<br>(0.49-1.07)<br>0.93     | 0               | 0              |
| <b>cT I (ng/dL)</b>      | 0-0.2            | 0.023±0.021<br>(0.006-0.092)<br>0.015                                                  | 0.01±0.002<br>(0.0001-0.0266)<br>0.011             | 0.021±0.007<br>(0.0021-0.0668)<br>0.011            | 0.0061             | 0.01, 0.02            | 0.02±0.006<br>(0.006-0.066)<br>0.016  | 0.01±0.002<br>(0.0001-0.0266)<br>0.011 | 0.0151±0.0026<br>(0.0001-0.0668)<br>0.0117         | 0               | 0              |

\*Ref: IDEXX (serum biomarkers), Saini (2014) (cT 1)

<sup>1</sup>Mean± SEM

<sup>2</sup>Figures in parentheses indicate range

<sup>3</sup>Median

Values in different rows with different alphabets differ significantly as compared to control at P ≤ 0.05

Dahlem et al. (2017) in a study of 95 dogs, observed significant higher levels of SDMA in acute kidney disease dogs and found it to be a noble biomarker which along with creatinine correlates strongly with GFR values.

In an experimental study on dogs, Donia et al., (2019) observed NGAL as early urinary biomarker for intense renal injury in canines. The rise in NGAL can be due to the fact that its upregulation in damaged proximal tubules incites re-epithelization of cells causing spike in its levels.

Supriya (2019) also reported positive correlation ( $r=0.54$ ) between serum SDMA and serum creatinine and reported positive correlation of cystatin C ( $r=0.68$ ) with serum creatinine.

#### **4.4.3.2 Urine biomarkers**

Mean levels of protein, UP: UC, uric acid, GGT, ALKP, SDMA, NGAL in urine were significantly higher in AKD dogs than of healthy group (Table 23).

Traditionally serum and urinary Cr were used as measure of GFR. However early changes in GFR can be predicted using various biomarkers. Biomarkers were prioritized to decrease reliance on serum creatinine level and urine output because these long-standing “gold-standard” functional markers are insensitive for the early recognition of kidney injury and real- time evaluation of AKI (Vijayan et al., 2016).

The renal tubular epithelial enzymes i.e.; GGT have been estimated in canine urine to anticipate cell spillage and early rounded injury or cell death (Brunker et al., 2009).

The urine protein: urine creatinine ratio is the easily accessible tool for diagnosis of renal origin proteinuria. Mean UP:UC in present study was around  $0.63 \pm 0.06$  (Table 23), indicating glomerular loss of protein (Welles et al., 2006). Glomerular loss of protein is suspected when mean UP:UC  $> 1$ . Higher UPC can also be seen in experimentally induced cystitis (Bagley et al., 1991). Results of higher urine protein were in accordance with study of Buranakarl et al. (2007) who found azotemic dogs with UPC  $> 1$ . Increased urine protein loss may be due to abnormal renal handling of protein by the kidneys during renal impairment causing renal proteinuria (Jaturakan et al., 2013).

ALKP is a brush-line enzyme and its level in urine has been related with proximal tubule harm in canines. Excretion of urinary soluble phosphatase is utilized to assess renal capacity in various conditions (Raekallio et al., 2006).

Kovarikova (2015) reported that urine GGT is ordinarily utilized for the discovery of intense kidney injury and observed a relationship between an increment in urinary NGAL and the improvement of AKD in canines. Perco et al. (2006) also reported positive correlation between urine NGAL and acute kidney injury in dogs.

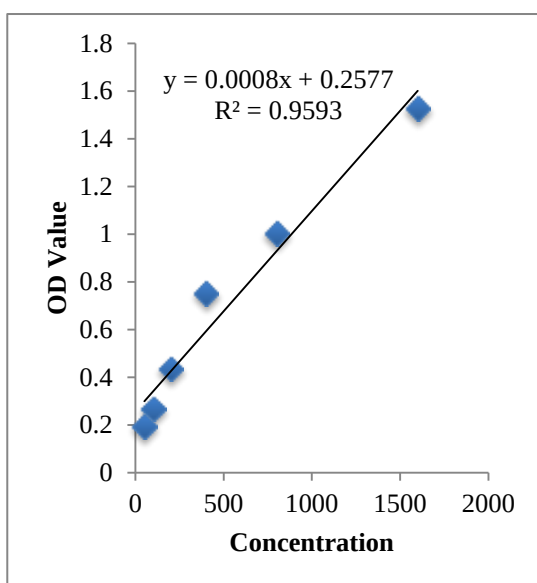


**Figure 6 (a): Sensitivity of Urine SDMA in ELISA**

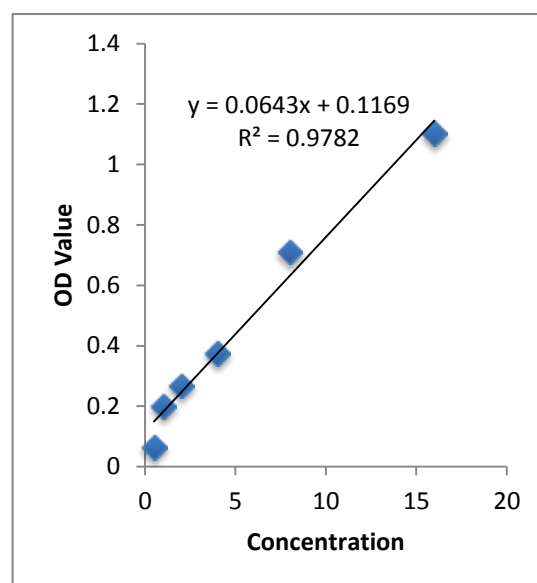


**Figure 6 (b): Sensitivity of Urine cystatin C in ELISA**

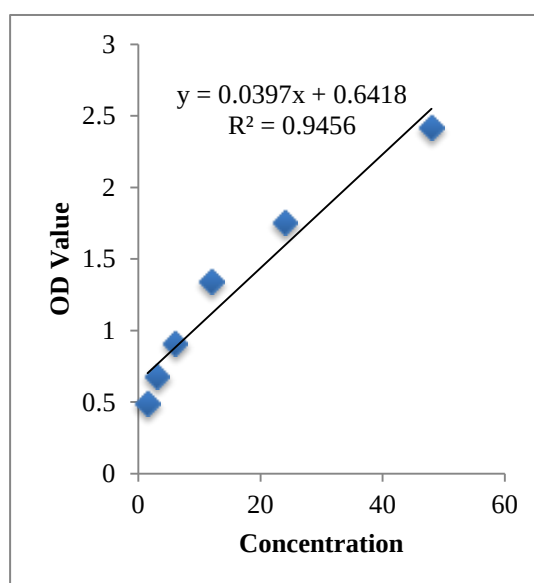


**Figure 6 (c): Sensitivity of Urine NGAL in ELISA**

Table 23: Urine renal biomarkers in dogs with acute kidney disease (n=20) as per IRIS staging

| Parameters               | Reference range* | Control (n=20)                                                               | Acute kidney disease                  |                                       |                                       | Overall (n=20)                                      | < Ref value (n) | >Ref value (n) |
|--------------------------|------------------|------------------------------------------------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------------------------|-----------------|----------------|
|                          |                  |                                                                              | Stage 2 (n=10)                        | Stage 3 (n=7)                         | Stage 4 (n=3)                         |                                                     |                 |                |
| <b>UP (mg/dL)</b>        | 6.5-8            | 6.96 <sup>A</sup> ±0.03 <sup>1</sup><br>(6-8) <sup>2</sup><br>7 <sup>3</sup> | 58.41±16.84<br>(3.1-188)<br>53        | 42.2±6.19<br>(22.1-65)<br>41          | 22.1±5.42<br>(11.4-29)<br>25.9        | 42.33 <sup>B</sup> ±6.14<br>(3.1-188)<br>35.1       | 1               | 19             |
| <b>UC (mg/dL)</b>        | 100-500          | 218.35 <sup>A</sup> ±4.61<br>(70-393)<br>198                                 | 93.17±19.40<br>(10.4-201)<br>78       | 71.04±16.71<br>(31-159)<br>52.3       | 56±13.79<br>(30-77)<br>61             | 91.44 <sup>B</sup> ±9.29<br>(10.4-201)<br>80        | 17              | 3              |
| <b>S Cr/UC</b>           | 0.01-0.02        | 0.0048 <sup>A</sup> ±0.0001<br>(0.001-0.014)<br>0.0035                       | 0.043±0.019<br>(0.009-0.211)<br>0.026 | 0.06±0.01<br>(0.02-0.12)<br>0.06      | 0.12±0.03<br>(0.07-0.2)<br>0.09       | 0.057 <sup>B</sup> ±0.070<br>(0.009-0.211)<br>0.038 | 1               | 19             |
| <b>UP:UC</b>             | 0-0.2            | .0393 <sup>A</sup> ±0.0012<br>(0.015-0.105)<br>0.035                         | 0.648±0.107<br>(0.046-1.057)<br>0.781 | 0.66±0.07<br>(0.40-0.91)<br>0.74      | 0.495±0.206<br>(0.148-0.863)<br>0.475 | 0.63 <sup>B</sup> ±0.06<br>(0.046-1.057)<br>0.734   | 0               | 20             |
| <b>Uric acid (mg/dL)</b> | 3.4-7.0          | 2.28 <sup>A</sup> ±0.02<br>(1.8-3.1)<br>2                                    | 4.5±0.49<br>(1.9-7)<br>4.45           | 4.67±0.45<br>(3.3-7)<br>2             | 5.96±1.12<br>(4-7.9)<br>6             | 4.78 <sup>B</sup> ±0.33<br>(1.9-7.9)<br>4.5         | 3               | 1              |
| <b>GGT (U/L)</b>         | 2.7-96.5         | 3.5 <sup>A</sup> ±0.03<br>(3-5)<br>3                                         | 18.8±8.62<br>(5-95)<br>10             | 31.14±19.36<br>(4-141)<br>6           | 73.66±36.149<br>(9-134)<br>78         | 31.35 <sup>B</sup> ±9.90<br>(4-141)<br>9            | 0               | 2              |
| <b>ALKP (U/L)</b>        | 10-150           | 8.69 <sup>A</sup> ±0.07<br>(4-10)<br>9                                       | 37.5±0.65<br>(16-61)<br>35            | 46.71±11.693<br>(15-101)<br>54        | 77±28.21<br>(21-111)<br>99            | 46.65 <sup>B</sup> ±1.99<br>(15-111)<br>41.5        | 0               | 0              |
| <b>SDMA (µg/dL)</b>      | 0-14             | 5.00 <sup>A</sup> ±0.16<br>(0.37-11.62)<br>4.75                              | 21.5±3.26<br>(11.62-39.12)<br>16.62   | 25.91±3.383<br>(15.37-37.87)<br>22.87 | 31.20±2.91<br>(26.62-36.62)<br>30.37  | 24.5 <sup>B</sup> ±2.13<br>(11.62-39.12)<br>24.12   | 0               | 17             |
| <b>NGAL (ng/mL)</b>      | 6-9              | 2.25 <sup>A</sup> ±0.09<br>(0.05-6.05)<br>2.22                               | 4.83±0.609<br>(0.634-7.506)<br>5.269  | 5.87±0.56<br>(3.15-7.53)<br>5.79      | 7.14±0.21<br>(6.72-7.46)<br>7.25      | 5.54 <sup>B</sup> ±0.39<br>(0.63-7.53)<br>5.81      | 11              | 0              |
| <b>Cystatin C (mg/L)</b> | 0.03-0.18        | 0.390±0.015<br>(0.0004-0.8881)<br>0.286                                      | 0.567±0.062<br>(0.289-0.818)<br>0.509 | 0.78±0.08<br>(0.38-1.06)<br>0.85      | 0.199±0.076<br>(0.098-0.348)<br>0.152 | 0.587±0.06<br>(0.098-1.06)<br>0.588                 | 1               | 19             |

\*Ref: IDEXX (urine biomarkers), Saini (2014) (cT 1); <sup>1</sup>Mean± SEM; <sup>2</sup>Figures in parentheses indicate range; <sup>3</sup>Median  
Values in different rows with different alphabets differ significantly at P≤0.05 as compared to control

**Table 24: Urine renal biomarkers in dogs with acute kidney disease (n=20) as per ACVIM and UP: UC staging**

| Parameters        | Reference range* | Control (n=20)                                                               | ACVIM staging                                       |                                                  |                    | UP:UC staging         |                                                      |                                                     | Overall (n=20)                                      | < Ref value (n) | >Ref value (n) |
|-------------------|------------------|------------------------------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------|--------------------|-----------------------|------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------------|-----------------|----------------|
|                   |                  |                                                                              | Normotensive (n=14)                                 | Pre hypertensive (n=5)                           | Hypertensive (n=1) | Non-proteinuric (n=2) | Mild proteinuric (n=6)                               | Proteinuric (n=12)                                  |                                                     |                 |                |
| UP (mg/dL)        | 6.5-8            | 6.96 <sup>A</sup> ±0.03 <sup>1</sup><br>(6-8) <sup>2</sup><br>7 <sup>3</sup> | 53.44±11.76<br>(11-188)<br>40.5                     | 35.1±13.29<br>(3.1-67)<br>29                     | 22.1               | 3.1, 11.4             | 42.33±7.81<br>(22-67)<br>35.5                        | 56.44±13.60<br>(11-188)<br>42                       | 42.33 <sup>B</sup> ±6.14<br>(3.1-188)<br>35.1       | 1               | 19             |
| UC (mg/dL)        | 100-500          | 218.35 <sup>A</sup> ±4.61<br>(70-393)<br>198                                 | 79.64±15.26<br>(10.4-201)<br>71.65                  | 88.4±17.93<br>(61-159)<br>77                     | 40                 | 67, 77                | 108.55±23.37<br>(61-200)<br>83                       | 66.80±14.36<br>(10.4-201)<br>50                     | 91.44 <sup>B</sup> ±9.29<br>(10.4-201)<br>80        | 17              | 3              |
| S Cr/UC           | 0.01-0.02        | 0.0048 <sup>A</sup> ±0.0001<br>(0.001-0.014)<br>0.0035                       | 0.05±0.01<br>(0.009-0.211)<br>0.03                  | 0.05±0.01<br>(0.02-0.09)<br>0.02                 | 0.105              | 0.032, 0.076          | 0.034±0.011<br>(0.009-0.090)<br>0.028                | 0.065±0.013<br>(0.018-0.211)<br>0.053               | 0.057 <sup>B</sup> ±0.070<br>(0.009-0.211)<br>0.038 | 1               | 19             |
| UP:UC             | 0-0.2            | 0.0393 <sup>A</sup> ±0.0012<br>(0.015-0.105)<br>0.035                        | 0.724 <sup>a</sup> ±0.064<br>(0.335-1.057)<br>0.802 | 0.387 <sup>b</sup> ±0.14<br>(0.046-0.85)<br>0.40 | 0.5525             | 0.046, 0.148          | 0.401 <sup>b</sup> ±0.023<br>(0.3350-0.475)<br>0.409 | 0.836 <sup>a</sup> ±0.037<br>(0.552-1.057)<br>0.848 | 0.63 <sup>B</sup> ±0.06<br>(0.046-1.057)<br>0.734   | 0               | 20             |
| Uric acid (mg/dL) | 3.4-7.0          | 2.28 <sup>A</sup> ±0.02<br>(1.8-3.1)<br>2                                    | 4.63±0.31<br>(2.9-7)<br>4.5                         | 4.74 <sup>a</sup> ±1.01<br>(1.9-7.9)<br>4        | 7                  | 1.9, 4.0              | 4.91 <sup>b</sup> ±0.75<br>(2.9-7.9)<br>4.6          | 5.01 <sup>a</sup> ±0.35<br>(3.3-7)<br>4.95          | 4.78 <sup>B</sup> ±0.33<br>(1.9-7.9)<br>4.5         | 3               | 1              |
| GGT (U/L)         | 2.7-96.5         | 3.5 <sup>A</sup> ±0.03<br>(3-5)<br>3                                         | 27.71±10.97<br>(4-141)<br>10.5                      | 46±26.05<br>(4-134)<br>8                         | 9                  | 8, 134                | 19.16 ±11.96<br>(4-78)<br>5.5                        | 30.83±12.60<br>(4-141)<br>10.5                      | 31.35 <sup>B</sup> ±9.90<br>(4-141)<br>9            | 0               | 2              |
| ALKP (U/L)        | 10-150           | 8.69 <sup>A</sup> ±0.07<br>(4-10)<br>9                                       | 46.28±6.005<br>(15-101)<br>47.5                     | 52.6±21.48<br>(16-111)<br>19                     | 22                 | 19, 111               | 49.33±12.09<br>(18-99)<br>47.5                       | 42.25±7.28<br>(15-101)<br>35                        | 46.65 <sup>B</sup> ±1.99<br>(15-111)<br>41.5        | 0               | 0              |
| SDMA (µg/dL)      | 0-14             | 5.00 <sup>A</sup> ±0.16<br>(0.37-11.62)<br>4.75                              | 24.30±2.44<br>(11.62-39.12)<br>24.12                | 22.37±4.68<br>(12.87-36.62)<br>16.62             | 37.875             | 12.87, 36.62          | 21.20±4.02<br>(12.87-36.62)<br>16.62                 | 26.10±2.56<br>(11.62-39.12)<br>26                   | 24.5 <sup>B</sup> ±2.13<br>(11.62-39.12)<br>24.12   | 0               | 17             |
| NGAL (ng/mL)      | 6-9              | 2.25 <sup>A</sup> ±0.09<br>(0.05-6.05)<br>2.22                               | 5.05±0.48<br>(0.63-7.53)<br>5.57                    | 6.51±0.50<br>(4.86-7.46)<br>7.15                 | 7.534              | 5.82, 7.46            | 5.57±0.67<br>(3.15-7.25)<br>5.92                     | 5.34±0.56<br>(0.63-7.53)<br>5.57                    | 5.54 <sup>B</sup> ±0.39<br>(0.63-7.53)<br>5.81      | 11              | 0              |
| Cystatin C (mg/L) | 0.03-0.18        | 0.390±0.015<br>(0.0004-0.8881)<br>0.286                                      | 0.65±0.07<br>(0.09-1.06)<br>0.67                    | 0.42±0.11<br>(0.15-0.85)<br>0.34                 | 0.384              | 0.343, 0.152          | 0.64±0.11<br>(0.28-0.92)<br>0.74                     | 0.61±0.07<br>(0.09-1.06)<br>0.58                    | 0.58±0.06<br>(0.09-1.06)<br>0.58                    | 1               | 19             |

\*Ref: IDEXX (urine biomarkers); <sup>1</sup>Mean± SEM; <sup>2</sup>Figures in parentheses indicate range; <sup>3</sup>Median

Values in different rows with different alphabets differ significantly at P≤0.05 as compared to control

## 4.5 Diagnostic imaging findings

### 4.5.1 Ultrasonography findings

Among various diagnostic aids in renal affections, ultrasound along with various biochemical parameters and relatable history provides useful tool for acute renal failure in dogs (Mannucci et al., 2019). Indications for renal ultrasonography include abnormal kidney size or shape (on palpation) or radiography along with signs of uraemia, haematuria or abdominal pain (Lamb, 1990). Canines typically have hyperechoic renal cortices. Ordinary renal cortical echogenicity in canines ought to be either isoechoic or hypoechoic to liver (Ivančić & Mai, 2008).

**Table 25: Ultrasonographic findings in dogs with acute kidney disease (n=19)**

| Ultrasound findings       | Acute kidney disease |                   |                  |                  | Total<br>(n=19)            |
|---------------------------|----------------------|-------------------|------------------|------------------|----------------------------|
|                           | Stage 2<br>(n=10)    | Stage 3<br>(n=12) | Stage 4<br>(n=7) | Stage 5<br>(n=1) |                            |
| Normal kidneys            | 7                    | 3                 | 2                | 0                | 12<br>(63.15) <sup>1</sup> |
| Hyperechoic renal cortex  | 2                    | 2                 | 1                | 1                | 6 (31.57)                  |
| Cystitis                  | 2                    | 1                 | 1                | 0                | 4 (21.05)                  |
| Medullary rim sign        | 0                    | 1                 | 1                | 1                | 3 (15.78)                  |
| Dilated renal pelvis      | 1                    | 1                 | 0                | 1                | 3 (15.7)                   |
| Ascites                   | 3                    | 0                 | 0                | 0                | 3 (15.2)                   |
| Renal cyst                | 0                    | 1                 | 1                | 0                | 2 (10.52)                  |
| Splenomegaly              | 1                    | 1                 | 0                | 0                | 2 (10.52)                  |
| Debris in urinary bladder | 1                    | 1                 | 0                | 0                | 2 (10.52)                  |
| Hydronephrosis            | 1                    | 1                 | 0                | 0                | 2 (10.52)                  |
| Distended urinary bladder | 1                    | 0                 | 0                | 0                | 1 (5.26)                   |
| Nephrolith                | 0                    | 1                 | 0                | 0                | 1 (5.26)                   |
| Hyperechoic medulla       | 0                    | 0                 | 1                | 0                | 1 (5.26)                   |
| Urethrolith               | 1                    | 0                 | 0                | 0                | 1 (5.26)                   |
| Renomegaly                | 0                    | 1                 | 0                | 0                | 1 (5.26)                   |
| Pyometra                  | 1                    | 0                 | 0                | 0                | 1 (5.26)                   |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 19

### Ultrasonography of abdomen photographs

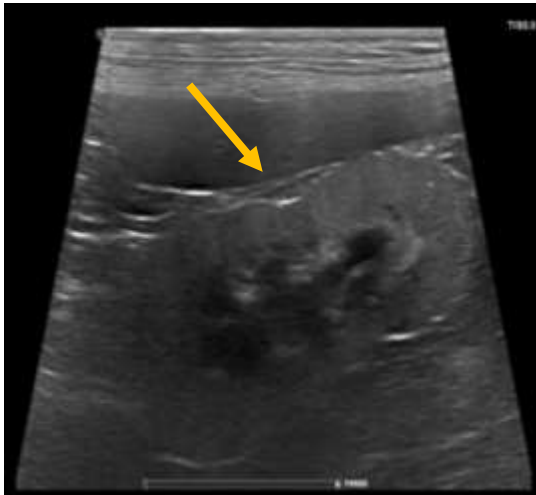


**Plate 6 (a): Increased cortical echogenicity**



**Plate 6 (b): Medullary rim sign**

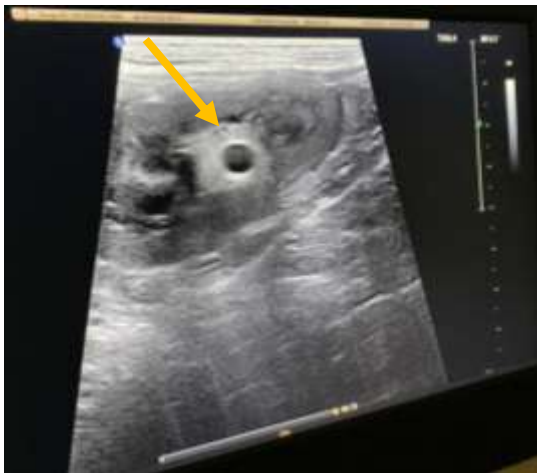


**Plate 6 (c): Renal cyst**

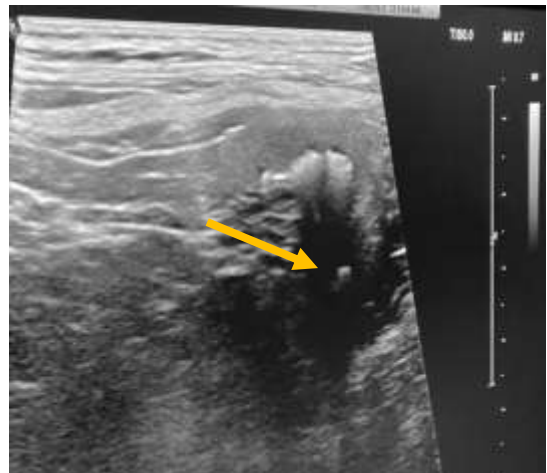


**Plate 6 (d): Nephrolith**

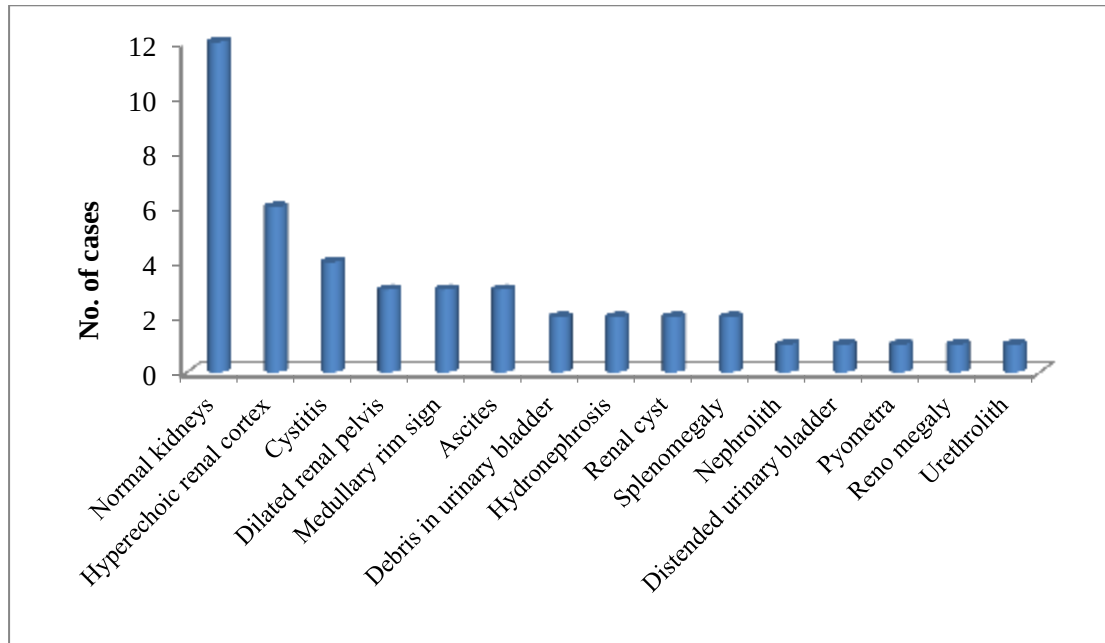


**Figure 7: Ultrasonographic findings in dogs with acute kidney disease (n=19)**

In present study, ultrasound was performed in 19 cases, out of which 12 were showing normal contour and echotexture (Table 25). Six cases showed increased cortical echogenicity. Two cases with NSAID toxicity showed cortical echogenicity along with medullary rim sign maintaining cortico-medullary differentiation indicating acute renal insult. Renomegaly found in one dog with anuria in present study may be due to compensatory hypertrophy, as seen in cases of leptospirosis or hydronephrosis (Stokes & Bartges, 2006). Changes in renal contour/echogenicity are probably due to cellular swelling, ischemia followed by cell death (Bartges & Polzin, 2011).

Dogs with medullary rim sign along with other related findings of renal insult have renal dysfunction (Mantis & Lamb, 2000). Doga (2006) observed mild nephrographic opacification, normal shape, size, maintenance of cortico-medullary differentiation and hyperechoic cortex in AKD. Stanley and Langston (2008) observed almost normal architecture and renal cortices with normal echogenicity in AKD. Bhojne (2016) observed nephrolith in 4 cases and concluded its presence to be an inciting cause of ARF or pyelonephritis.

Supriya (2019) observed alterations in the renal parenchyma (n=36) having hyperechoic renal cortex when compared to the hepatic and splenic parenchyma, complete loss of differentiation between the renal cortex and medulla (n=15) and nephrolith (n=2).

#### 4.5.2 Radiographic findings

According to Chew et al. (2011), radiography is considered to be a useful tool to access kidney's shape, size, growth and fluid.

Out of 30 dogs with AKD, radiography was done in 8 cases (Table 26) out of which 4 were having arthritis, 2 cases each of cardiomegaly, interstitial lung pattern, bronchial lung pattern, cystitis and one of splenomegaly.

Arthritis/spondylosis can be an inciting cause of ARF due to increased levels of pro- inflammatory mediators (TNF-a, interleukin-1b, interleukin-6) and cytokines (IL-10) leading to microvasculature damage of kidneys (Ramesh & Reeves, 2004).

**Table 26: Radiographic findings in dogs affected with Acute kidney disease (n=8)**

| Condition                    | Acute kidney disease |               |               |               | Total (n) |
|------------------------------|----------------------|---------------|---------------|---------------|-----------|
|                              | Stage 2 (n=5)        | Stage 3 (n=1) | Stage 4 (n=0) | Stage 5 (n=0) |           |
| Arthritis                    | 3                    | 1             | 0             | 0             | 4         |
| Cardiomegaly                 | 2                    | 0             | 0             | 0             | 2         |
| Interstitial pattern in lung | 2                    | 0             | 0             | 0             | 2         |
| Bronchial pattern            | 2                    | 0             | 0             | 0             | 2         |
| Cystitis/thick bladder       | 1                    | 1             | 0             | 0             | 2         |
| Splenomegaly                 | 1                    | 0             | 0             | 0             | 1         |

#### 4.6 Urine analysis

##### 4.6.1 Gross examination

Complete urine assessment requires evaluation of various types of cells (RBCs, WBCs and epithelial cells), organisms, casts, crystals, mucosal threads/shedding's, etc.

Miller et al. (1978) stated that urinary indices are of great value in assessing the renal function. Serum BUN and Cr along with routine urine analysis provides an important tool for early diagnosis of renal insufficiencies. Analysis of urine (quality, quantity) is important in assessing AKD.

In present study, colour of urine was pale yellow in 16 cases, dark yellow in 11 cases and reddish tinged in 3 cases (Table 27). Normal urine produced is transparent, yellow or amber. Colour intensity is related to the volume and the

concentration of urine. Pale yellow urine is normal or may indicate a polyuric state. Red, brown, or black urine suggests the presence of blood, haemoglobin, myoglobin, or bilirubin (Callens & Bartges, 2015).

Urine specific gravity was < 1.015 in 17 dogs and 1.015-1.030 in 13 dogs. Kidneys with normal functioning retains specific gravity in range from 1.018 to 1.025 (Chew & DiBartola, 1998). Dogs with early signs of renal dysfunction possess weak urine concentrating ability. Specific gravity <1.025 with clinical dehydration indicates reduced renal functioning (Chew et al., 2011). Cowgill and Francey (2005) concluded that ARF diagnosis can be made when azotemia is accompanied by isosthenuria (USG 1.015-1.030) or with minimally concentrated urine (USG < 1.008) in canines.

**Table 27: Urinalysis in dogs affected with acute kidney disease as per IRIS staging (n=30)**

| Parameters                             |                               | Acute kidney disease |                   |                  |                  | Total<br>(n)            |
|----------------------------------------|-------------------------------|----------------------|-------------------|------------------|------------------|-------------------------|
|                                        |                               | Stage 2<br>(n=10)    | Stage 3<br>(n=12) | Stage 4<br>(n=7) | Stage 5<br>(n=1) |                         |
| Macroscopic examination                |                               |                      |                   |                  |                  |                         |
| Color                                  | Dark yellow                   | 4                    | 4                 | 2                | 1                | 11 (36.66) <sup>1</sup> |
|                                        | Pale yellow                   | 4                    | 7                 | 5                | 0                | 16 (53.33)              |
|                                        | Reddish                       | 2                    | 1                 | 0                | 0                | 3 (10)                  |
| Odor                                   | Ammonical                     | 1                    | 0                 | 0                | 0                | 1 (3.33)                |
|                                        | Nil                           | 9                    | 12                | 7                | 1                | 29 (96.66)              |
| Turbidity                              | Present                       | 0                    | 0                 | 0                | 0                | 0 (0)                   |
|                                        | Absent                        | 10                   | 12                | 7                | 1                | 30 (1)                  |
| Strip test                             |                               |                      |                   |                  |                  |                         |
| pH                                     | Acidic (5-7)                  | 7                    | 8                 | 4                | 1                | 20 (66.66)              |
|                                        | Neutral (7)                   | 2                    | 3                 | 2                | 0                | 7 (23.33)               |
|                                        | Alkaline (>7)                 | 1                    | 1                 | 1                | 0                | 3 (10)                  |
| Specific gravity<br>(by refractometer) | <1.015<br>(hyposthenuria)     | 7                    | 7                 | 3                | 0                | 17 (56.66)              |
|                                        | 1.015-1.030<br>(Isosthenuria) | 5                    | 4                 | 3                | 1                | 13 (43.33)              |
|                                        | >1.030<br>(hypersthenuria)    | 0                    | 0                 | 0                | 0                | 0 (0)                   |

| Parameters              |                                         | Acute kidney disease |                |               |               | Total (n)  |
|-------------------------|-----------------------------------------|----------------------|----------------|---------------|---------------|------------|
|                         |                                         | Stage 2 (n=10)       | Stage 3 (n=12) | Stage 4 (n=7) | Stage 5 (n=1) |            |
| Bilirubin               | 1+ (0.4 mmol/L)                         | 2                    | 1              | 0             | 0             | 3 (10)     |
|                         | 2+ (0.6 mmol/L)                         | 0                    | 0              | 1             | 0             | 1 (3.33)   |
| Blood                   | 1+ (10 cells/ $\mu$ L)                  | 1                    | 3              | 1             | 0             | 5 (16.66)  |
|                         | 2+ (25 cells/ $\mu$ L)                  | 2                    | 1              | 3             | 0             | 6 (20)     |
|                         | 3+ (80 cells/ $\mu$ L)                  | 2                    | 0              | 1             | 0             | 3 (10)     |
| Protein                 | 1+ (0.3 g/L)                            | 3                    | 5              | 3             | 0             | 11 (33.3)  |
|                         | 2+ (1 g/L)                              | 2                    | 3              | 1             | 0             | 6 (20)     |
|                         | 3+, 4+ (3, 6 g/L)                       | 0                    | 0              | 1             | 0             | 1 (3.33)   |
| Urobilinogen            | 0.2 $\mu$ mol/L                         | 1                    | 1              | 0             | 0             | 2 (6.66)   |
| Microscopic examination |                                         |                      |                |               |               |            |
| Cells                   | Renal Epithelial cells (>4-6 cells/hpf) | 3                    | 1              | 4             | 1             | 9 (30)     |
|                         | Urothelial cells (>4-6 cells/hpf)       | 6                    | 2              | 4             | 0             | 12 (40)    |
|                         | Red Blood cells (>2-4 cells/hpf)        | 2                    | 0              | 2             | 1             | 5 (16.66)  |
|                         | WBC/Pus cells (>4-8 cells/hpf)          | 0                    | 0              | 2             | 1             | 3 (10)     |
| Casts and crystals      | Granular casts                          | 4                    | 2              | 3             | 1             | 10 (33.33) |
|                         | Waxy casts                              | 1                    | 0              | 4             | 1             | 6 (20)     |
|                         | RBC casts                               | 0                    | 0              | 0             | 1             | 1 (3.33)   |
|                         | Hyaline casts                           | 3                    | 0              | 5             | 0             | 8 (26.66)  |
|                         | Granular + Waxy casts                   | 0                    | 2              | 0             | 0             | 2 (6.66)   |
|                         | Phosphate crystals                      | 4                    | 0              | 1             | 0             | 5 (16.66)  |
|                         | Calcium oxalate crystal                 | 1                    | 1              | 0             | 0             | 2 (6.66)   |
|                         | Bilirubin crystals                      | 1                    | 0              | 1             | 0             | 2 (6.66)   |
| Urine culture positive  |                                         | 4                    | 5              | 3             | 1             | 13 (43.33) |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30

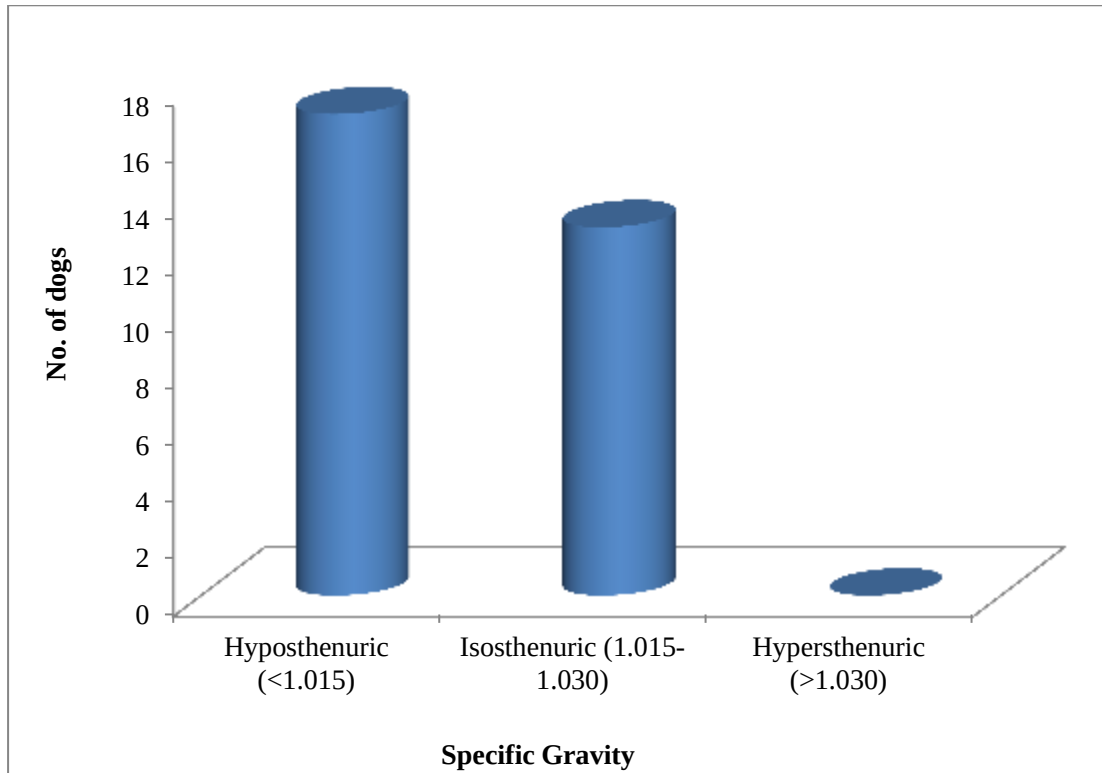


**Figure 8: Urine specific gravity (Refractometer) in dogs affected with acute kidney disease (n=30)**

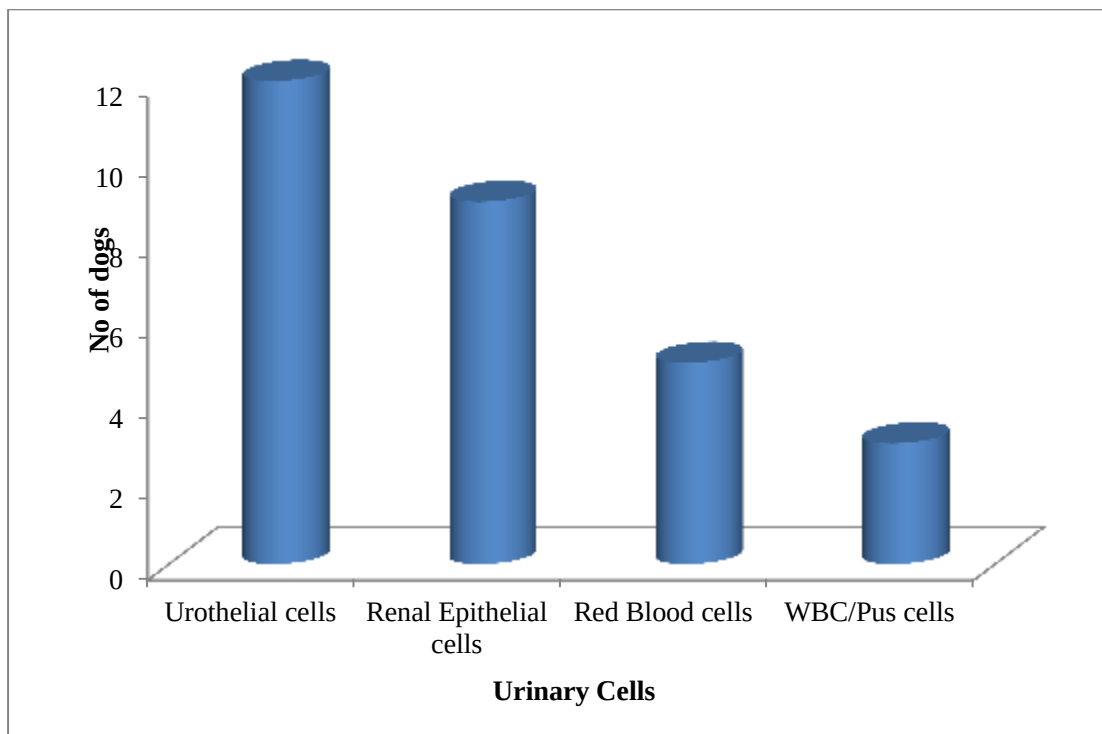


**Figure 9: Microscopic examination (cells) in urine in dogs affected with acute kidney disease (n=30)**

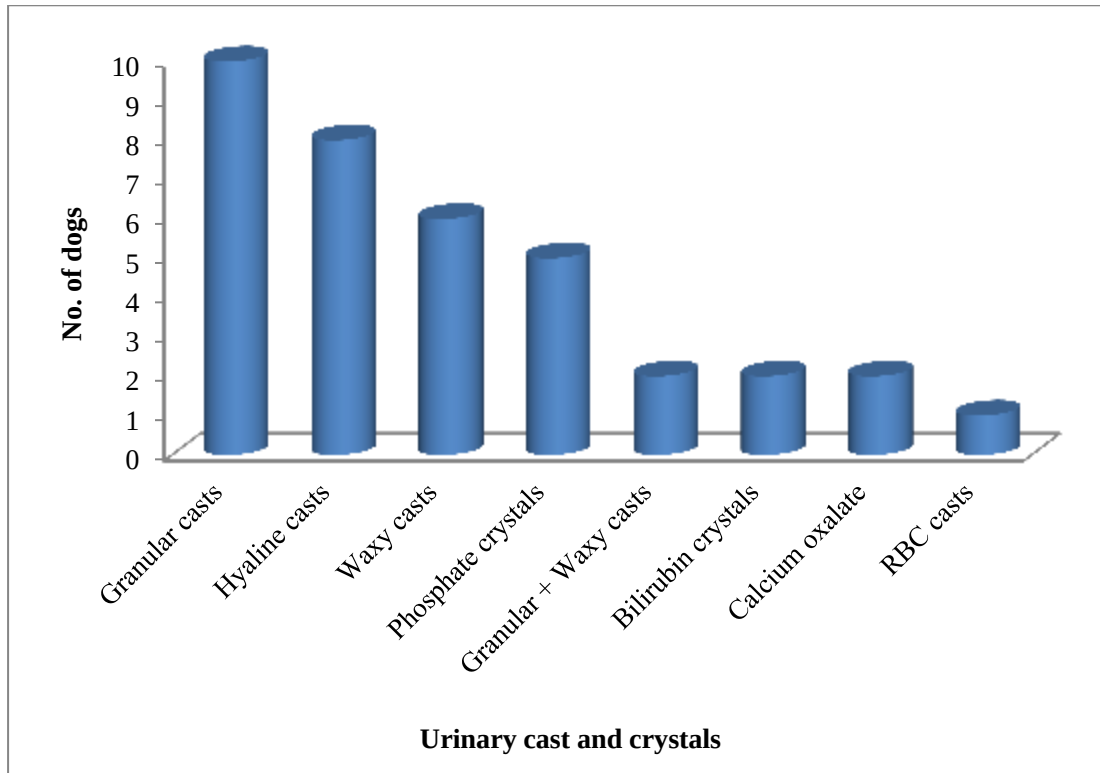


**Figure 10: Microscopic examination (casts and crystals) of urine in dogs affected with acute kidney disease (n=30)**

#### 4.6.2 Chemical examination of urine

Qualitative chemical examination of urine samples was done with dipstick analysis and observed 1+ proteinuria in 33 per cent dogs, 2+ in 20 per cent and 1+ in 3 per cent AKD cases (Table 27). Proteinuria was quantitatively observed in 18 out of 20 AKD dogs indicating probable glomerular loss of protein (Welles et al., 2006). Renal proteinuria is frequently observed in dogs affected with the renal failure or having varied uremia irrespective of the etiology (Jaturakan et al., 2013).

Bilirubinuria observed was 1+ in 3 dogs and 2+ in one dog. The possible causes for bilirubinuria may be haemolysis, IMHA, liver dysfunction (secondary), EHBO, pyrexia, starvation, etc. (Chew et al., 2011).

On microscopic examination of urine samples, different type of cells observed in urine in AKD dogs were renal epithelial cells (n=9), urothelial cells (n=12), RBC's (n=5), WBC's (n=3). Pyuria is an important finding in infectious diseases of the kidneys or urinary tract and is commonly observed in canine renal failure (Latimer, 2003). RBC's in urine of renal failure dogs was also reported by Latimer (2003). The

## Urine microscopic examination

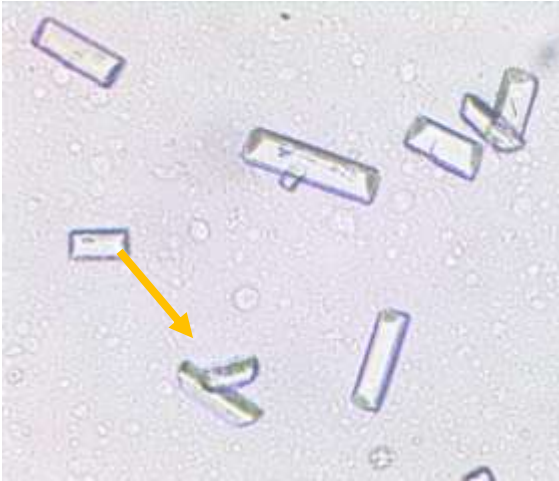


**Plate 7 (a): Triple phosphate crystals in urine on microscope examination (40X)**



**Plate 7 (b): Hyaline cast in urine on microscope examination (40X)**



**Plate 7 (c): Bilirubin cast in urine on microscope examination (40X)**



**Plate 7 (d): RBC's in urine on microscope examination (40X)**

presence of RBCs in the urine of canines especially in renal and post-renal conditions indicates infection of the kidneys or urinary tract. Urothelial cells are insignificant in predicting any of the renal illness but its increased number along with epithelial cells may be indicative of ischemic, degenerative or nephrotoxic renal injury (Chew et al., 2011).

Casts and crystals observed on microscopic examination were granular cast (n=10), waxy cast (n=6), hyaline cast (n=8), granular + waxy cast (n=2) and crystals viz., phosphate (n=5) and calcium oxalate crystals (n=2) (Table 27, Figure 10). Increased granular cast may be seen in nephritic syndrome whereas hyaline cast indicates renal insufficiency associated with proteinuria (Nelson & Couto, 2014).

Tufani et al. (2017) concluded that hyaline casts are protein precipitates commonly seen in glomerular diseases. Excessive granular casts suggest accelerated tubular degeneration and may be seen in glomerular disease. Few waxy casts presented in some cases with renal insufficiency are suggestive of substantial chronic intra-renal stasis.

Out of 90 cases of renal failure, Supriya (2019) observed that 16 dogs had renal epithelial/urothelial cells, 9 had increased number of RBCs and 39 dogs had pus cells in the urine. Among cast and crystals, 19 dogs had granular casts, 24 had waxy casts and 2 had hyaline cast.

#### **4.6.3 Urine culture and sensitivity test**

In present study, urine samples from AKD dogs were tested for bacterial culture and sensitivity. *E. coli* was found in 4 dogs, *Staphylococcus sp.* in 3, *Proteus sp.* in 2 and *Streptococcus sp.* in 1. *E. coli* was most sensitive to ceftriaxone-sulbactam followed by amoxicillin-clavulanic acid and ampicillin. *Staphylococcus sp.* were most sensitive to enrofloxacin followed by amoxicillin. *Proteus sp.* was sensitive to only ciprofloxacin. *Streptococcus sp.* was sensitive to ceftriaxone-sulbactam and amoxicillin (Table 28).

Normally the bladder and kidneys remain sterile whereas distal urethra, perpetual cavity and peri-vaginal covering in females are prominent source of commensals (Nelson and Couto, 2014). Athaley et al. (2018) observed *Escherichia coli* as predominant organism followed by *Pseudomonas aeruginosa* (16.67%),

*Staphylococcus aureus* (8.33%) and *Klebsiella pneumonia* (2.78%) in dogs. Tufani et al. (2017) also observed *Escherichia coli* as predominant organism in 38.10% dogs followed by *Staphylococcus aureus* (20.63%), *Proteus spp.* (17.46%) and *Streptococcus spp.* (6.35%).

On CST of urine from 90 dogs with renal failure, Supriya (2019) isolated *Staphylococcus* (n=16) followed by *E. coli* (n=9), *Streptococcus* (n=3), *E. coli* and *Staphylococcus* (n= 2 each), *Staphylococcus* and *Streptococcus* (n=2 each). *E. coli* were most sensitive to ceftriaxone, followed by amoxicillin, enrofloxacin, doxycycline, and ciprofloxacin. *Staphylococci* were most sensitive to ciprofloxacin than amoxicillin, ceftriaxone, ampicillin, metronidazole, enrofloxacin and doxycycline. *Streptococci* were most sensitive to ampicillin as compared to other antibiotics.

**Table 28: Urine culture and antibiotic sensitivity test in dogs affected with AKD (n=10)**

| Bacteria                        | CIP | AMX | CEFT-SB | AMP | ENR | AMX-CL | GEN | MET |
|---------------------------------|-----|-----|---------|-----|-----|--------|-----|-----|
| <i>E. coli</i> (n=4)            | R   | R   | S       | S   | R   | S      | R   | R   |
| <i>Staphylococcus sp.</i> (n=3) | R   | S   | R       | R   | S   | R      | S   | R   |
| <i>Proteus sp.</i> (n=2)        | S   | R   | S       | R   | R   | R      | R   | R   |
| <i>Streptococcus sp.</i> (n=1)  | R   | S   | S       | R   | R   | R      | R   | R   |

\*CIP (ciprofloxacin), AMX (amoxicillin), CEFT-SB (ceftriaxone-sulbactam), AMP (ampicillin), ENR (enrofloxacin), AMX- CL (amoxicillin-clavulanic acid), GEN (gentamicin), MET (metronidazole).

#### 4.7 Hemoprotozoan and rickettsial infection

For the isolation of various hemoprotozoan, whole blood samples from 30 AKD dogs were stained with Leishman stain. Blood samples from these dogs were also subjected to DNA isolation. PCR was employed and amplicons of various hemoprotozoan viz., *B. canis vogeli* (601 bp) and *E. canis* (380 bp) were observed.

On blood smear examination, only two out of 30 samples were found to be positive for *Ehrlichia canis*. On PCR analysis, five samples were positive which included *Babesia canis vogeli* in one (3.3%) case and *Ehrlichia canis* in 4 cases

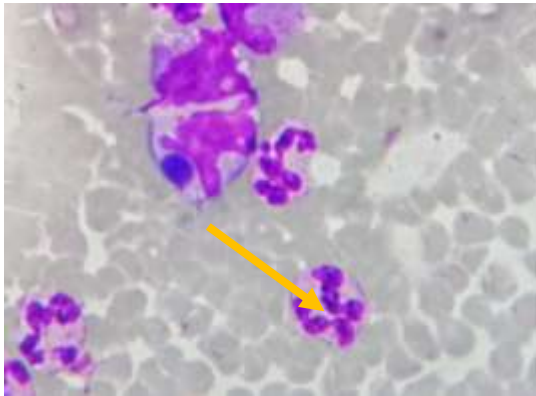


**Plate 8 (a): *E. canis* on blood smear  
Examination (100X)**



**Plate 8 (b): *B. canis* on blood smear  
Examination (100X)**

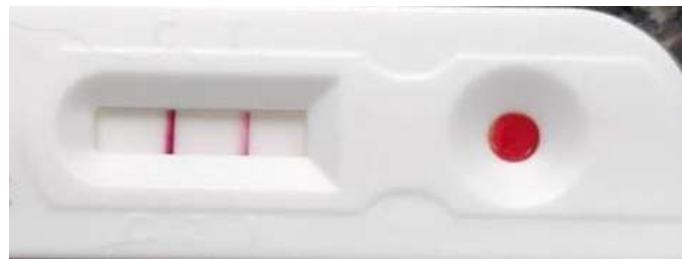


**Plate 8 (c): *E. canis* positive on spot on test kit**

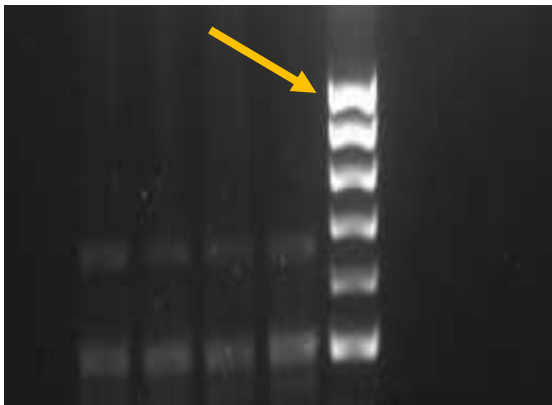


**Plate 8 (d): *B. canis vogeli* (601 bp- Arrow)  
on PCR**

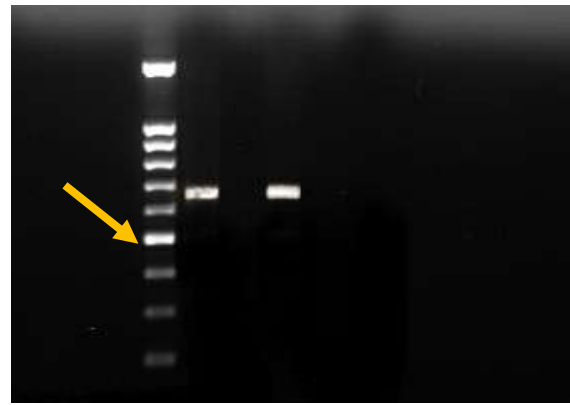


**Plate 8 (e): *E. canis* (380 bp-Arrows) on  
PCR**

(13.3%). For hemoprotozoan detection, PCR assay was found to be more specific than slide smear method (Table 29).

Supriya (2019) observed that out of 90 cases of kidney diseases 5 were positive on blood smear examination for hemoprotozoan and rickettsia. On PCR analysis different hemoprotozoan and rickettsia observed were *Babesia gibsoni* (n=11), *Babesia canis vogeli* (n=2), *Ehrlichia canis* (n=1), *Hepatozoon canis* (n=11) cases while mixed infection of *E. canis* and *H. canis* (n=1).

Singh et al. (2014) observed overall prevalence of 7.47 per cent for canine babesiosis using Leishman stain while molecular diagnosis revealed *B. gibsoni* in 15.42 percent cases indicating higher sensitivity of PCR as compared to smear examination.

Immune complex deposition in glomerular basement membrane and vasculitis with or without acute tubular necrosis have been proposed as cause of hemoprotozoan induced acute nephropathy (Camacho et al., 2004).

**Table 29: Hemoprotozoan and rickettsia infection on blood smear examination and PCR analysis in dogs with acute kidney disease (n=30)**

| <b>Hemoprotozoan and Rickettsia</b> | <b>Positive on blood smear examination (n<sub>1</sub>=2)</b> | <b>Positive on PCR (n<sub>2</sub>=5)</b> |
|-------------------------------------|--------------------------------------------------------------|------------------------------------------|
| <i>Babesia canis</i>                | 0 (0)                                                        | 1 (3.33) <sup>1</sup>                    |
| <i>Ehrlichia canis</i>              | 2 (6.66)                                                     | 4 (13.33)                                |
| <b>Total</b>                        | <b>2 (6.66)</b>                                              | <b>5 (16.66)</b>                         |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30

## **4.8 Post-mortem examination**

### **4.8.1 Gross examination**

Post mortem examination was performed in 2 dogs who died during course of study. One dog had died after 1 week of symptomatic treatment and other before commencement of the treatment. Splenomegaly along with renal cyst was observed in first dog (Plate 10 a-d) and hypertrophied right kidney & necrotic bladder wall was observed in second dog with anuria.

Enlarged kidney could be due to compensatory hypertrophy, as seen in cases of leptospirosis or hydronephrosis (Stokes & Bartges, 2006). Sludge/debris may promote UTI and may cause localised bladder irritation and thus necrotic wall/cystitis (Nelson & Couto, 2014). Splenomegaly can either be due to sequel of hemoprotozoan disorder or extra medullary hematopoiesis in dog. Sharma et al (2016) found enlarged spleen on necropsy in a 3 year old dog with *Ehrlichiosis*. Solitary renal cyst can either be non-pathological or may induces AKD via renal cystogenesis causing progressive ischemia-reperfusion injury (IRI) (Kurbegovic & Trudel, 2016). Bhojne (2016) found renal cortical cyst (n=4) and renomegaly (n=12) in an epidemiological study of renal failure in 9149 dogs.

#### **4.8.2 Histopathology**

Histopathological examination of renal tissues was done in both the cases presented with the history of vomition, lethargic, anuria and fever. H & E stained smear revealed cellular infiltration in tubular regions and glomerular tuft in first dog with vomition, lethargic and swelling around glomeruli, damage to basement membrane were seen along with glomerular thickening in second (anuric) suggestive of glomerulonephritis (Plate 10 e-f).

Eubig et al. (2005) observed moderate tubular degeneration in PCT, cellular debris in dilated lumens and basement membrane damage in 16 dogs with grape ingestion. In a study over 184 dogs, histopathology of 6 dogs revealed acute tubular necrosis to marked cortical necrosis (Langenberg et al., 2008).

#### **4.9 Probable etiology of acute kidney disease**

In present study, the probable etiologies in dogs affected with AKD were: undefined etiology (n=10, 33.33%), cystitis (n= 4, 13.33%), arthritis (n=3, 10%), *E canis*, NSAID intoxication, pyometra, ascites (n=2 each, 6.6%) and *B canis*, ascites+ *E canis*, septic wound, IMHA+ *E. canis*, post-surgery (n=1 each, 3.33%), etc.



**Plate 9 (a): Right hypertrophied kidney on post-mortem examination**



**Plate 9 (b): Cortico-medullary cyst on post-mortem examination**



**Plate 9 (c): Splenomegaly on post-mortem examination**



**Plate 9 (d): Necrotic urinary bladder on post-mortem examination**

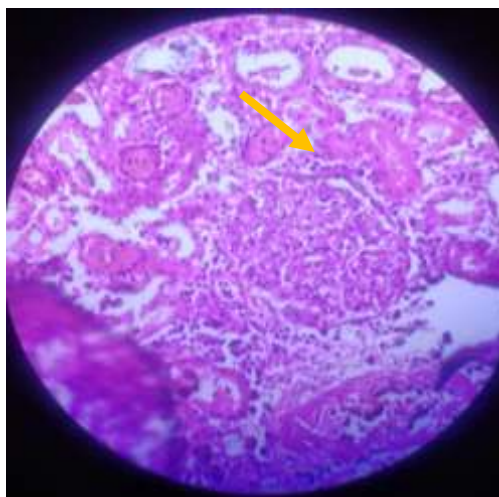


**Plate 9 (e): Glomerular swelling on microscopic examination (100X)**

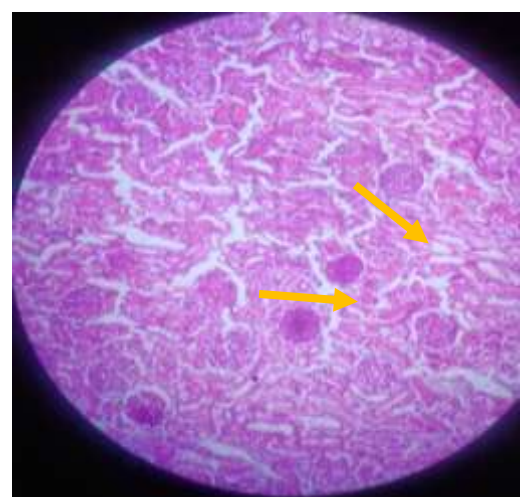


**Plate 9 (f): Tubular and glomerular cellular infiltration on microscopic examination (100X)**

**Table 30: Probable etiology in dogs with acute kidney disease (n=30)**

| <b>Probable Etiology</b>        | <b>Cases (N=30)</b>   |
|---------------------------------|-----------------------|
| <i>Babesia canis</i>            | 1 (3.33)              |
| <i>Ehrlichia canis</i>          | 2 (6.66) <sup>1</sup> |
| Ascites+ <i>Ehrlichia canis</i> | 1 (3.33)              |
| IMHA+ <i>Ehrlichia canis</i>    | 1 (3.33)              |
| Pyometra                        | 2 (6.66)              |
| NSAID intoxication              | 2 (6.66)              |
| Ascites                         | 2 (6.66)              |
| Cystitis                        | 4 (13.33)             |
| Septic wound                    | 1 (3.33)              |
| Arthritis                       | 3 (10)                |
| Post-surgery                    | 1 (3.33)              |
| Not Defined                     | 10 (33.33)            |

<sup>1</sup>Figures in parentheses indicate percentage of dogs out of 30

#### **4.10 Management of dogs with acute kidney disease**

Out of 30 cases of AKD, 26 cases were managed with conventional therapy and 4 cases were treated with IHD along with conventional treatment. Conventional treatment included supportive and symptomatic treatment with fluid therapy, broad spectrum antibiotics, diuretics, antacids, antiemetics, Reno protectants, supportive care, etc. as per the requirement of the case and suspected underlying cause (Table 9).

##### **4.10.1 Intermittent hemodialysis (IHD)**

IHD was performed in 4 dogs with AKD. Animals were assessed by hematological and biochemical parameters both before and after dialysis (Table 31). The patients being ambulatory received single session of dialysis except 1 dog (with the history of snake ingestion) in which 3 sessions were done. In 1<sup>st</sup> session the aim was to reduce urea/urea like substances by approximately 25 per cent over 60 minutes. The urea reduction ratio (URR%) and creatinine reduction ratio (CrRR%) were measured after dialysis session and were used to assess the efficacy of therapy (Sherman et al., 1995). Ideally these ratios should be around 65 per cent in successive sessions. In present study, the overall mean CrRR was 28.85± 5.86 per cent, URR was 36.8±15.9 per cent and total mean blood volume processed during 1<sup>st</sup> session was 155.36±42.57 ml/kg. Lower reduction rates are due shorter duration of first session.

The mean serum creatinine levels before dialysis was 10.35±3.32 mg/dl and post dialysis was 7.25±3.46 mg/dl, while mean BUN levels were 115.33±36.87 mg/dl and 76.25±28.25 mg/dl, respectively. In dog with 3 sessions, mean serum creatinine levels decreased from 20.2 to 11 mg/dl in 3<sup>rd</sup> session with normal regain of feed intake and no apparent signs of illness. However, the dog collapsed due to owner's non-compliance to standard treatment protocols. Two dogs were fine and living quality life till last follow up (upto 4 months post-treatment) and one dog collapsed after 1 month of treatment.

Pelander et al. (2015) stated that refractory azotemia (BUN > 90 mg/dl, serum creatinine >6mg/dl), acute renal insult, hyperkalaemia, fluid overload, severe metabolic acidosis, acute poisoning or drug overdose, preoperative conditioning for renal transplantation and post-operative delayed graft function are all indications for veterinary hemodialysis.

When serum urea nitrogen concentrations surpass 90 mg/dl (32 mmol/l) and serum creatinine concentrations exceed 8 mg/dl (700 mol/l), hemodialysis is most likely to be effective (Segev et al., 2016).

Patients with severe uremia who receive hemodialysis have a longer life expectancy and a greater window of opportunity for recovery if done in initial stages of AKI with minimal renal damage. Severity or progression towards chronic stage requires IHD as a life prolongation step with poor prognosis. For etiology that can be managed medically/conventional treatment, IHD had almost similar outcomes (Segev et al., 2008).

**Table 31: Intermittent haemodialysis treatment in dogs with acute kidney disease (n=4)**

| Case no.          | Creatinine (mg/dl) |                   | CrRR%              | BUN (mg/dl)          |                     | URR%              | Blood processed (ml/kg) |
|-------------------|--------------------|-------------------|--------------------|----------------------|---------------------|-------------------|-------------------------|
|                   | Pre-dialysis       | Post-dialysis     |                    | Pre-dialysis         | Post-dialysis       |                   |                         |
| 1                 | 9.4                | 7.2               | 23.40              | 89                   | 77                  | 13.4              | 57.77                   |
| 2                 | 20                 | 17                | 15                 | 180                  | 155                 | 13.8              | 121.7                   |
| 3                 | 6.6                | 1.4               | 40                 | >140                 | 26                  | 81.4              | 185.8                   |
| 4                 | 5.4                | 3.4               | 37                 | 77                   | 47                  | 38.9              | 256.2                   |
| <b>Mean ± S.E</b> | <b>10.35± 3.32</b> | <b>7.25± 3.46</b> | <b>28.85± 5.86</b> | <b>115.33± 36.87</b> | <b>76.25± 28.25</b> | <b>36.8± 15.9</b> | <b>155.36± 42.57</b>    |



**Plate 10 (a): Dialysis tubing's and catheter**



**Plate 10 (b): Central Venous Catheter placement (pre-dialysis)**



**Plate 10 (c): Intermittent Hemodialysis in dog**



**Plate 10 (d): Working dialysis apparatus**

**Table 32: Haematological and biochemical analysis during pre-treatment and post-treatment of dogs affected with acute kidney disease (n=20)**

| Parameters                                            | Reference range | Overall renal failure                                                   |                                                          |
|-------------------------------------------------------|-----------------|-------------------------------------------------------------------------|----------------------------------------------------------|
|                                                       |                 | Pre-treatment                                                           | Post-treatment                                           |
| <b>Hb (g/dL)</b>                                      | 12-18           | 11.39±0.78 <sup>1</sup><br>(3.9-16.2) <sup>2</sup><br>12.3 <sup>3</sup> | 11.2±0.48<br>(6.2-14.3)<br>11.1                          |
| <b>TEC (×10<sup>6</sup> per mm<sup>3</sup>)</b>       | 5.5-8.5         | 5.25±0.29<br>(2.09-7.14)<br>5.71                                        | 5.424±0.23<br>(3.34-6.94)<br>5.40                        |
| <b>PCV (%)</b>                                        | 37-55           | 34.71±2.04<br>(13.9-50)<br>35.7                                         | 34.03±1.45<br>(18.5-43.2)<br>33.95                       |
| <b>Platelet (×10<sup>3</sup> per mm<sup>3</sup>)</b>  | 200-800         | 223.85±36.32<br>(18-729)<br>223.5                                       | 248.95±30.02<br>(69-544)<br>216.5                        |
| <b>TLC (per mm<sup>3</sup>)</b>                       | 6000-17000      | 20795.5±4024.6<br>(2400-90100)<br>18170                                 | 14717±2327<br>(5980-55400)<br>12717                      |
| <b>Absolute Neutrophil count (per mm<sup>3</sup>)</b> | 3000-115000     | 15033.95 <sup>A</sup> ±1785.79<br>(1296-28710)<br>16795.8               | 8418.01 <sup>B</sup> ±646.72<br>(3946.8-13125)<br>9744   |
| <b>Absolute Lymphocyte Count (per mm<sup>3</sup>)</b> | 1000-4800       | 2550.85 <sup>A</sup> ±272.12<br>(1104-4723)<br>2353.4                   | 4008.80 <sup>B</sup> ±385.12<br>(2033.2-7056)<br>3136.84 |
| <b>N:L</b>                                            | 2.1-8.84        | 9.7856 <sup>A</sup> ±2.38<br>(1.173-49)<br>6.1428                       | 2.3808 <sup>B</sup> ±0.17<br>(1.3809-4)<br>2.2795        |
| <b>BUN (mg/dL)</b>                                    | 8.8-25.9        | 44.15 <sup>A</sup> ±11.02<br>(13-240)<br>28.5                           | 16.9 <sup>B</sup> ±1.67<br>(8-35)<br>15.5                |
| <b>Cr (mg/dL)</b>                                     | 0.5-1.4         | 3.0965 <sup>A</sup> ±0.31<br>(1.7-6)<br>2.55                            | 1.31 <sup>B</sup> ±0.083<br>(0.7-2)<br>1.2               |
| <b>USG</b>                                            | 1.015-1.030     | 1.0155 <sup>A</sup> ±0.0012<br>(1.009-1.026)<br>1.015                   | 1.025 <sup>B</sup> ±0.0013<br>(1.018-1.035)<br>1.02      |

\*Ref: Textbook of Small Animal Practice Saunders 3<sup>rd</sup> edition (2006)

<sup>1</sup>Mean± SEM

<sup>2</sup>Figures in parentheses indicate range

<sup>3</sup>Median

Values in different rows with different alphabets differ significantly at  $P \leq 0.05$  as compared to control

#### 4.10.2 Conventional therapy

Out of 30 dogs presented with acute kidney injury, 26 dogs were treated symptomatically with fluid therapy, broad spectrum antibiotics, diuretics, antacids, anti-emetics, vitamin, mineral support, renal protectants, etc. (Table 9).

Antimicrobials are used in leptospirosis, pyelonephritis, sepsis, endocarditis, or any rickettsial disorders (Labato, 2001). All animals were essentially maintained on omega-3 and Heckler which promotes mesangial circulation and inhibits inflammatory migrants.

Fluid therapy was used according to the clinical signs and the actual assessment of dehydration status with emphasis on promoting diuresis and renal hemodynamics. Schetz (2004) emphasised continuous use of diuretics (furosemide) rather than bolus in AKI and increases significant urine output significantly. Mannitol was used in 4 dogs inducing diuresis and significant results were seen. Mannitol has additional effects of free radical scavenging and improving glomerular circulation (Bagshaw et al., 2007).

Patient's positive for hemoprotozoan diseases (n=5) were treated with oral doxycycline (@10mg/kg P.O, OD) and assessed after 4 weeks. They showed significant improvement in hemato-biochemical parameters.

Two dogs with NSAID intoxication (Aceclofenac and Meloxicam) were continuously monitored and nephrotoxic drug use was strictly prohibited. Additionally with Reno protectant, alkaliser, fluid therapy and diuresis were given. Animals showed significant improvement after treatment was continued with omega-3, Heckler tablets for about 1 month. Continuous use of NSAID's may cause interstitial nephritis by papillary necrosis (Hunter et al., 2011).

Out of 26 cases, 20 dogs recovered during course of treatment and 6 dogs died within a course of one week. Out of these 6 dogs, one dog had IMHA+ *E canis*, second had low Hb, third had ascites and fourth had *B canis vogeli*, all died within 3 days, while other 2 with undefined etiology died within a week of start of treatment.

All dogs which survived were still receiving omega-3 and Heckler tablets and living quality life till last follow up upto 60 days from start of treatment. Among them, 4 dogs had creatinine within range but at higher end (1.6mg/dl).

Twenty dogs that survived after treatment were monitored periodically for various hemato-biochemical parameters (Table 32). TLC count in dogs with acute kidney disease reduced non-significantly post treatment. After treatment a significant decline in BUN and creatinine levels were recorded in all the surviving dogs.

Mann (2013) treated 46 renal failure dogs conservatively using fluids, antiemetics antacids, broad spectrum antibiotics and diuretics. With dietary intervention, 10 dogs showed significant improvement in BUN, creatinine and phosphorous levels. Bellomo et al. 1993 concluded IHD to be effective in cases refractory to either symptomatic treatment or unable to maintain normal urine output despite all treatment measures.

Supriya (2019) treated 90 renal failure cases with conventional and symptomatic therapy, after treatment 11 (12.22%) dogs recovered with normal renal function tests and remaining 79 (87.78%) dogs died at different time intervals.

## CHAPTER – V

### SUMMARY AND CONCLUSIONS

The present study entitled **“A study on early diagnosis of acute kidney disease and its therapeutic management in dogs”** was undertaken at the Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana with the objectives: (i) To undertake early diagnosis of acute kidney disease in dogs; (ii) To evaluate treatment with or without hemodialysis in acute kidney disease in dogs.

In the first objective dogs presented with renal insufficiency irrespective of sex, breed and age with signs of anorexia, inappetance, vomiting, oliguria, anuria, lethargy, melena, halitosis, etc of  $\leq 7$  days duration were selected for the diagnosis of underlying renal insufficiency. A total of 156 cases with above mentioned criteria were screened, out of which 77 cases were having Cr levels below 1.6 mg/dL. Out of remaining dogs, dogs with serum creatinine levels of  $\geq 1.6$  mg/dL and BUN  $\geq 25$  mg/dL were selected for further investigation. Out of remaining 79 cases, 49 cases were in CRF and the remaining 30 dogs of renal insufficiency/failure which were having characteristics of Acute Kidney Disease were selected for further investigation to identify the underlying cause. These 30 dogs were classified as per IRIS guidelines. IRIS Grade 1: non-azotemic AKI (Cr  $<1.6$  mg/dl), Grade 2: mild AKI (Cr 1.7 to 2.5 mg/dl, n=10), Grade 3: moderate to severe azotemic (Cr 2.6–5 mg/dl, n=12), Grade 4: moderate to severe azotemic (Cr 5.1–10 mg/dl, n=7) and Grade 5: severe azotemic (Cr  $>10$  mg/dl, n=1).

Twenty apparently healthy dogs presented for routine examination, deworming, vaccination and general health check up with apparently normal physical examination findings were selected as a control group for comparative study.

Thirty cases of AKD were subjected to detailed investigations including history and physical examination. Blood and urine samples were collected and subjected to analysis of CBC, LFT, RFT, glucose, cholesterol, minerals, electrolytes, serum renal biomarkers, routine urinalysis, urine renal biomarkers, urine CST, hemoprotozoa & rickettsia examination, etc. They were also subjected to diagnostic aids like radiology and ultrasonography, etc. as per requirement. Above investigations were undertaken to identify the cause of AKD and to decide treatment.

The second objective was to undertake the treatment of these 30 cases of AKD with either conventional therapy alone (n=26) or combination of conventional and intermittent hemodialysis (n=4).

The Mean age of the dogs affected with AKD was  $5.22 \pm 0.51$  years. The maximum dogs were in the age group of 0-4 years (n=15, 50%), followed by 4-8 years (n=12, 40%) and 8-12 years (n=3, 10%). More cases were in male dogs (63.33%) than female (37.66%). Labrador retrievers were found to be more affected (36.6%) followed by Pug (10%) and Pitbull (10%). Major clinical entities observed were inappetance (76.65%) followed by vomition (63.33%), lethargy (43.33%). Other signs included fever, retching, halitosis, diarrhoea and melena. The mean rectal temperature ( $101.79 \pm 0.29^\circ\text{F}$ ) did not vary significantly from control group. Heart rate ( $121.16 \pm 4.33/\text{min}$ ) was significantly lower than control group. Respiration rate varied from 14-110/min, yet mean respiration rate was not significantly different from the control group. Mean Hb, PCV, TEC levels were significantly lower in AKD dogs than the control group. Mean TLC, absolute neutrophil & lymphocyte counts and N:L were significantly higher than control group indicating that AKD may be associated with infection. Overall Neutrophil to lymphocyte (N:L) ratio was higher among all groups of renal failure as compared to the control group dogs. BUN and creatinine levels were significantly ( $P \leq 0.05$ ) higher and BUN/ Cr lower in AKD dogs as compared to the control group. Mean  $P_i$  levels,  $\text{Cr}/P_i$  were significantly higher and  $\text{Ca}/P_i$  significantly lowers in AKD dogs.

There was no significant change in  $\text{K}^+$ , Mg, glucose, ALT and TP in AKD dogs. AST, ALKP, GGT, cholesterol and fibrinogen levels were significantly higher in AKD dogs while albumin was significantly lower than control dogs.

The mean levels of serum SDMA, NGAL and Cystatin C were significantly higher as compared to the control group. Pearson correlation also showed moderate positive correlation of SDMA with serum creatinine levels.

Mean levels of protein, UP:UC, uric acid, GGT, ALKP, SDMA, NGAL in urine were significantly higher in AKD dogs as compared to healthy group. Mean UP:UC was around  $0.63 \pm 0.06$ .

Ultrasound was performed in 19 cases, out of which 12 were showing normal contour and echotexture. Six cases showed increased cortical echogenicity. Two cases

with NSAID toxicity showed increased cortical echogenicity along with medullary rim sign maintaining cortico-medullary differentiation. Renomegaly found in one dog with anuria. Radiography was performed in 8 cases out of which 4 were having arthritis, 2 cases each had cardiomegaly, interstitial lung pattern, bronchial lung pattern, cystitis and one had splenomegaly.

Colour of urine was pale yellow in 16 cases followed by dark yellow in 11 and reddish tinged in 3 cases. Urine specific gravity was < 1.015 in 17 dogs and 1.015-1.030 in 13 dogs. On microscopic examination of urine, different type of cells observed were renal epithelial cells (n=9), urothelial cells (n=12), RBC's (n=5) and WBC's (n=3). Casts and crystals observed were granular cast (n=10), waxy cast (n=6), hyaline cast (n=8), granular + waxy cast (n=2) and crystals of phosphate (n=5) and calcium oxalate (n=2). Urine samples were also tested for bacterial culture and sensitivity. *E. coli* was found in 4 dogs, *Staphylococcus sp.* in 3, *Proteus sp.* in 2 and *Streptococcus sp.* in 1. *E. coli* was most sensitive to ceftriaxone-sulbactam followed by amoxicillin-clavulanic acid and ampicillin. *Staphylococcus sp.* were most sensitive to enrofloxacin followed by amoxicillin. *Proteus sp.* was most sensitive to only ciprofloxacin. *Streptococcus sp.* was sensitive to ceftriaxone-sulbactam and amoxicillin.

On blood smear examination only two out of 30 samples were found positive for *Ehrlichia canis*. On PCR analysis, five samples were positive which included *Babesia canis vogeli* in one (3.3%) and *Ehrlichia canis* in 4 cases (13.3%).

Postmortem examination was performed in 2 dogs with the history of renal dysfunction (vomiting, inappetance, lethargic), out of which one dog had died after 1 week of symptomatic treatment and other before commencement of the treatment. Splenomegaly along with renal cyst was observed in first dog, while hypertrophied right kidney and necrotic bladder wall was observed in second dog with anuria. Histopathological examination revealed cellular infiltration in tubular regions and glomerular tuft in first dog, Swelling around glomeruli and damage to basement membrane along with glomerular thickening were seen in second dog suggestive of glomerulonephritis.

In present study, the probable etiology of dogs affected with AKD was of undefined etiology (n=10, 33.33%) followed by cystitis (n= 4, 13.33%), arthritis (n=3,

10%), *E. canis*, NSAID intoxication, pyometra, ascites (each n=2, 6.6%) and *B. canis*, ascites+ *E. canis*, septic wound, IMHA+ *E. canis*, post-surgery (each n=1, 3.33%), etc.

Out of 30 cases of AKD, 26 were managed with conventional therapy and 4 cases were treated with intermittent hemodialysis (IHD) along with conventional treatment. Conventional treatment included supportive and symptomatic treatment with fluid therapy, broad spectrum antibiotics, diuretics, antacids, antiemetics, Reno-protectants, supportive care, etc. as per the requirement of the case and suspected underlying cause.

Three dogs which were managed with IHD, received single session of dialysis while 1 dog (with the history of snake ingestion) received 3 sessions. In first session the aim was to reduce urea/urea like substances by approximately 25% over 60 minutes. The urea reduction ratio (URR%) and creatinine reduction ratio (CrRR%) were measured after dialysis session and used to assess the efficacy of therapy. Ideally These ratios should be around 65% in successive sessions. The overall mean CrRR was  $28.85 \pm 5.86\%$ , URR was  $36.8 \pm 15.9\%$  and total mean blood volume processed during first session was  $155.36 \pm 42.57 \text{ ml/kg}$ . Lower reduction rates are due shorter duration of first session. The mean serum creatinine level before dialysis was  $10.35 \pm 3.32 \text{ mg/dl}$  and post dialysis was  $7.25 \pm 3.46 \text{ mg/dl}$ , while mean BUN level was  $115.33 \pm 36.87 \text{ mg/dl}$  and  $76.25 \pm 28.25 \text{ mg/dl}$ , respectively. In dog with 3 sessions, mean serum creatinine levels decreased from 20.2 to 11 mg/dl in 3<sup>rd</sup> session with normal regain of feed intake and no apparent signs of illness. However, the dog collapsed due to owner's non-compliance of follow up treatment. Two dogs were fine and living quality life till last follow up (upto 4 months post-treatment) and one dog collapsed after 1 month of treatment.

Twenty six cases managed with conventional treatment which included fluid therapy, broad spectrum antibiotics, diuretics, antacids, anti-emetics, vitamin, mineral support, renal protectants, etc. Patient's positive for hemoprotozoan diseases were treated with oral doxycycline (@10mg/kg P.O, OD) and assessed after 4 weeks. They showed significant improvement in hemato-biochemical parameters. Two dogs with NSAID intoxication (Acelofenac and meloxicam) were continuously monitored and nephrotoxic drug use was strictly prohibited. Additionally Reno-protectant, alkaliser, fluid therapy and diuresis were also given. Animals showed significant improvement

after treatment. Out of these 26 cases, 20 dogs recovered during course of treatment and 6 dogs died within a course of one week. Out of these 6 dogs, one dog had IMHA+ *E canis*, second had low Hb, third had ascites and fourth had *B canis vogeli*, which died within 3 days and other 2 with undefined etiology died within a week. Twenty dogs that survived after treatment were monitored periodically for various hemato-biochemical parameters. TLC count decreased non-significantly post treatment. After treatment a significant decline in BUN and creatinine levels were recorded in all the surviving dogs.

## Conclusions

- Out of 30 dogs with AKD as per IRIS guidelines, 10 dogs are in IRIS grade 2: mild AKI (Cr 1.7 to 2.5 mg/dl), 12 in grade 3: moderate to severe azotemic (Cr 2.6–5 mg/dl), 7 in grade 4: moderate to severe azotemic (Cr 5.1–10 mg/dl) and one in grade 5: severe azotemic (Cr >10 mg/dl). Twenty apparently healthy dogs were selected as a control group for comparative study.
- Maximum dogs with AKD are in the age group of < 4 years (n=15, 50%), followed by 4-8 years (n=12, 40%) and 8-12 years (n=3, 10%).
- Mean Hb, PCV, TEC levels are significantly lower while mean TLC, absolute neutrophil & lymphocyte counts and N:L are significantly higher in AKD dogs than the control group.
- Mean BUN, Cr, P<sub>i</sub> and Cr/P<sub>i</sub> levels are significantly ( $P \leq 0.05$ ) higher and BUN/Cr & Ca/P<sub>i</sub> lower in AKD dogs than control group.
- Mean levels of serum SDMA, NGAL and Cystatin C are significantly higher in AKD dogs.
- Out of 19 cases in which ultrasonography was performed, 12 showed normal contour & echotexture, 6 cases showed increased cortical echogenicity and 2 cases with NSAID toxicity showed increased cortical echogenicity along with medullary rim sign. Renomegaly was found in one dog with anuria.
- Urine specific gravity is < 1.015 (hypostenuric) in 17 dogs and 1.015-1.030 (Isothenuric) in 13 dogs. On microscopic examination of urine, different type of cells observed are renal epithelial cells (n=9), urothelial cells (n=12), RBC's (n=5) and WBC's (n=3). Casts and crystals observed are granular cast (n=10),

waxy cast (n=6), hyaline cast (n=8), granular + waxy cast (n=2) and crystals of phosphate (n=5) and calcium oxalate (n=2). Urine samples were also tested for bacterial culture and sensitivity. *E. coli* is found in 4 dogs, *Staphylococcus sp.* in 3, *Proteus sp.* in 2 and *Streptococcus sp.* in 1.

- On blood smear examination 2 cases are found positive for *Ehrlichia canis*, while on PCR analysis, 5 dogs are positive for hemoprotozoa (*Babesia canis vogeli*, n=1) and rickettsia (*Ehrlichia canis*, n= 4).
- Postmortem examination was performed in 2 dogs with the history of renal dysfunction (vomiting, inappetance, lethargic), out of which one dog died after 1 week of symptomatic treatment and other before commencement of the treatment. Splenomegaly along with renal cyst is observed in first dog, while hypertrophied right kidney and necrotic bladder wall is observed in second dog with anuria. Histopathology in 2 dogs suggested glomerulonephritis.
- The probable etiology of dogs affected with AKD is of undefined etiology (n=10, 33.33%) followed by cystitis (n= 4, 13.33%), arthritis (n=3, 10%), *E. canis*, NSAID intoxication, pyometra, ascites (each n=2,6.6%) and *B. canis*, ascites+ *E. canis*, septic wound, IMHA+ *E. canis*, post-surgery (each n=1, 3.33%), etc.
- Out of 4 dogs which were managed with IHD along with conventional treatment, three dogs received single session of dialysis and they showed mean CrRR of  $28.85 \pm 5.86$  per cent, URR of  $36.8 \pm 15.9$  per cent and total mean blood volume processed during first session is  $155.36 \pm 42.57$  ml/kg. Two dogs were fine and living quality life till last follow up (upto 4 months post-treatment) and one dog collapsed after 1 month of treatment.
- Out of 26 cases which were managed with conventional treatment, 20 dogs recovered during course of treatment and 6 dogs died within a course of one week. After treatment a significant decline in BUN and creatinine levels are recorded in all the surviving dogs.

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