CLINICOPATHOLOGICAL STUDIES OF CANINE RENAL DYSFUNCTION WITH SPECIAL REFERENCE TO LIPOCALIN AND BETA 2-MICROGLOBULIN BIOMARKERS

THESIS

Submitted

in partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PATHOLOGY

BY
LOTHE KALYANI MADHUKARRAO
Enrolment No.: V/10/064

Bombay Veterinary College, Mumbai

MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY, NAGPUR – 440 001
(INDIA)

2018

Appendix - B

DECLARATION OF THE STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"CLINICOPATHOLOGICAL STUDIES OF CANINE RENAL DYSFUNCTION WITH SPECIAL REFERENCE TO LIPOCALIN AND BETA 2-MICROGLOBULIN BIOMARKERS"** or part thereof has not been submitted for any of the other degree or diploma of any university, nor the data have been derived from any thesis or publications of any university or scientific organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

Date- / /2018

Signature
(LOTHE KALYANI MADHUKARRAO)
Enrolment No. V/10/064
Reg. No. 1573

Date- / /2018

Signature

(Dr. D. P. KADAM)
Counter signed by
Chairman, Advisory Committee with date
Professor of Veterinary Pathology
Bombay Veterinary College

Appendix – C (i)

DECLARATION OF ADVISORY COMMITTEE

LOTHE KALYANI MADHUKARRAO has satisfactorily prosecuted her course of research work for a period of not less than one semester and that the thesis entitled **"CLINICOPATHOLOGICAL STUDIES OF CANINE RENAL DYSFUNCTION WITH SPECIAL REFERENCE TO LIPOCALIN AND BETA 2-MICROGLOBULIN BIOMARKERS"**submitted by her is the result of research work is sufficient to warrant its presentation to the examination in subject of **VETERINARY PATHOLOGY** for the award of **MASTER OF VETERINARY SCIENCE (M.V.Sc.)** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by her for a degree of any other university.

Place: Mumbai
Date:

Dr. D. P. KADAM
Advisor / Guide
Professor,
Department of Veterinary Pathology

ADVISORY COMMITTEE
Name and Signature of Advisory Committee Members

Sr.No.	Name	Designation	Signature
1	Dr. S. D. Moregaonkar (Member)	Professor Department of Veterinary Pathology, COVAS, Parbhani	
3	Dr. R. R. Pharande (Member)	Assistant Professor of Veterinary Microbiology, Bombay Veterinary College, Parel, Mumbai-400012.	
4	Dr.C. N. Galdhar (Member)	Assistant Professor of Veterinary Clinical Medicine, Bombay Veterinary College,Parel,Mumbai-400012	
5	Dr. P. M. Kekan (Member)	Assistant Professor, Department of Veterinary Physiology, Bombay Veterinary College,Parel,Mumbai-400012	

Appendix – C (ii)

CERTIFICATE

This is to certify that the thesis entitled **"CLINICOPATHOLOGICAL STUDIES OF CANINE RENAL DYSFUNCTION WITH SPECIAL REFERENCE TO LIPOCALIN AND BETA 2-MICROGLOBULIN BIOMARKERS"** submitted by **LOTHE KALYANI MADHUKARRAO** to the Maharashtra Animal Sciences University, Nagpur, in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

(Dr. D. P. KADAM)

Name & Signature of External Examiner | **Head of the Department** | **Advisor / Guide Professor, Dept. of Veterinary Pathology**

Sr.No.	Name	Designation	Signature
1	Dr. S. D. Moregaonkar (Member)	Professor Department of Veterinary Pathology, COVAS, Parbhani	
3	Dr. R. R. Pharande (Member)	Assistant Professor of Veterinary Microbiology, Bombay Veterinary College, Parel, Mumbai-400012.	
4	Dr.C. N. Galdhar (Member)	Assistant Professor of Veterinary Clinical Medicine, Bombay Veterinary College,Parel,Mumbai-400012	
5	Dr. P. M. Kekan (Member)	Assistant Professor, Department of Veterinary Physiology, Bombay Veterinary College,Parel,Mumbai-400012	

Date:

Signature with seal
Dean / Associate Dean
Bombay Veterinary College

ACKNOWLEDGEMENT

I at the verge of becoming Master in Veterinary pathology will definitely choke in my chores if I do not recognize and acknowledge all the good hearts that were instrumental throughout my work. There are a number of stratum without whom this thesis might have not been written and to whom I am greatly indebted.

First of all I would like to thank **God** for granting me ability and knowledge to complete my thesis successfully. I consider myself fortuitously and greatly privileged to articulate my profound sense of gratitude and scot and lot from core of my heart to my research guide **Dr. D. P. Kadam, Ph.D.,** Professor, Bombay Veterinary College, Mumbai. He let me proliferate independently as a researcher and also drawn out his helping hand whenever indispensable. Also, his fosterage and belief in my abilities contributed greatly to the success not only in my study but also in my personal life.

I express my deep sense of gratitude and heartfelt thanks to member of Advisory committee, **Dr. S. D. Moregaonkar, Ph.D.,** Professor, Department of Veterinary Pathology, COVAS Parbhani, for his pragmatic suggestion, expert guidance and pertinent comments and succored me a lot during the study period by helping in planning the research work.

I am profoundly indebted to the venerated members of my advisory committee **Dr. C. N. Galdhar**, Assistant Professor of Department of Veterinary Clinical Medicine, **Dr. R. R. Pharande**, Assistant Professor of Department of Veterinary Microbiology, **Dr. P. M. Kekan**, Assistant Professor, Department of Veterinary Physiology, for their timely guidance and technical advice in carrying out the research work.

My special thanks to **Dr. P. V. Meshram, Dr. D. S. Dhaygude** and **Dr. P. Gadhave** Assistant Professor**,** Department of Veterinary Pathology, Bombay Veterinary College, Mumbai, for their relentless efforts, enlightened guidance, continuous support, encouragement, time and help with all aspects of work undertaken during the research work.

I place on record my sincere thanks and gratitude to **Dr. K. V. Garud** for his help rendered during the course of study.

I deeply acknowledge my colleagues **Dr. Trisha** and **Dr. Biswajit** for their constant support & help during my post graduation and research work. Without them this journey was not possible. I feel Blessed to have completed my post graduation with them and wish them a grand success for their future endeavors.

I would like to express my sincere thanks to my beloved senior **Dr. Dyaneshwar Gavane, Dr. Aniket Mhase, Dr. Reshma Patil** and **Dr. Sayali Ramteke**, for their help during my P.G. studies & research work.

A special thanks to my juniors **Dr.Roshani, Dr. Madhulika, Dr. Pooja, Dr. Radhika** and **Dr. Nivrutti** for giving scientific support and wish them a grand success for future.

I am thankful to **Dr. Suryawanshi** who helped me during my post graduation & research work. I also want to thank **Shri. Lahu kaka** who helped me a lot during my post graduation.

I express my sincere thanks to my friends **Dr. Archana, Dr. Shraddha, Dr. Priyanka, Dr. Ashwini, Dr. Vrushali, Dr. Santosh, Dr. Anil, Dr. Prasad**, **Dr. Renuka** and **Dr. Dhanashree.** We spent most important years of our life together and shared our joys and sorrows. I feel very fortunate to have such great friends and memories of the time spent together will be there with me forever. I also want to thank my senior friends **Dr. Netra** for her help in my research work. I also want to thank my special friend **Dhanush** for his tremendous support and understanding throughout my post graduation.

I surrender my regards, thanks, love and affection to my parents **Mr. Madhukar Lothe** and **Mrs. Alka Lothe** who showered dote and philia, which made me to remember that, there is always a place to look back and say "I have home". I want to dedicate this thesis to my late mother **Mrs. Urmila Lothe** who always wanted her daughters to be self-sufficient **"Thanks Aai"**. I also want to thank my siblings **Manjusha, Sharvari, Aniket and Harshal** for keeping child inside me awake.

Last but certainly not the least, thanks to my beloved college, Nagpur Veterinary College and Bombay veterinary College. All credit goes to these heavens of education which makes me who I am.

I extend my heartfelt gratitude to all those that might have not been mentioned here in haste, yet they will always be remembered for their help.

Place: Mumbai
Date: / / 2018

(LOTHE KALYANI MADHUKARRAO)

TABLE OF CONTENTS

LISTOF TABLES

LIST OF FIGURES

LIST OF PLATES

17	Tubular degeneration and coagulative necrosis (H & E; 10X)		:	82-83
18	A	Multifocal areas of tubular degeneration and infiltration of MNC in interstitial tissue (H & E; 10 X)	:	82-83
	B	Infiltration of MNC (Plasma cells) (H & E; 40 X)		
19	A	Adhesion of glomerulus with Bowman's capsule (H & E; 40 X)	:	82-83
	B	Diffuse tubular cellular swelling and tubular coagulative necrosis (H & E; 10 X)		
	C	Fibrous tissue proliferation in glomerulus and interstitial tissue (Masson's Trichrome stain; 40 X)		
20	Multifocal tubular degeneration (H & E; 10 X)		:	82-83
21	A	Thickening of Bowman's capsule (H & E; 40 X)	:	82-83
	B	Tubular degeneration and necrosis		
22	Tubular degeneration and fibrosis (H & E; 10 X)		:	82-83
23	Multifocal cystic dilatation tubules, infiltration of MNC and fibrosis (H & E; 10 X)		:	82-83
24	Diffuse cystic dilatation of tubules and Multifocal infiltration of MNC (H & E; 10 X)		:	82-83
25	A	Hyalinization of glomerulus (H & E; 40 X)	:	82-83
	B	Tubular necrosis and fibrous connective tissue proliferation in interstitial tissue (H & E; 10 X)		
26	Multifocal tubular degeneration (H & E; 10 X)		:	82-83
27	A	Infiltration of MNC (Plasma cells) (H & E; 40 X)	:	82-83
	B	Diffuse tubular degeneration and severe infiltration of MNC (H & E; 40 X)		
28	Multifocal areas of tubular coagulative necrosis and degeneration (H & E; 40 X)		:	82-83
29	A	Multifocal glomerular atrophy and calcification at Bowman's capsule (H & E; 40 X)	:	82-83
	B	Typical tubular calcification and degeneration (H & E; 40 X)		
	C	Calcification shown in black colour (Von kossa stain; 10 X)		
30	Degeneration and congestion (H & E; 40 X, 10 X)			82-83
31	Atrophy of chords, degeneration and sinusoidal dilatation (H & E; 40 X)			82-83

LIST OF ABBREVIATIONS

Sr. No.	Abbreviations		Name
1	%	:	Percentage
2	ALB	:	Albumin
3	ALP	:	Alkaline Phosphatase
4	ARF	:	Acute Renal Failure
5	AKI	:	Acute Kidney Injury
6	B2M	:	Beta 2 microglobulin
7	BID or DB	:	Direct bilirubin
8	BIT or TB	:	Total bilirubin
9	BUN	:	Blood Urea Nitrogen
10	CD	:	Critical Difference
11	CKD	:	Chronic Kidney Disease
12	CREAT		Serum Creatinine
13	CRF	:	Chronic Renal Failure
14	cumm		Cubic millimeter
15	df	:	degree of freedom
16	dl	:	Deciliter
17	ELISA		Enzyme Linked Immunosorbent Assay
18	ELFA		Enzyme Linked Fluourescent Assay
19	F cal	:	F calculated
20	fl		Femtoliter
21	g or gm	:	Gram
22	GLB		Globulin
23	Hb	:	Hemoglobin
24	HRP	:	Horse Redox Peroxidase
25	IB		Indirect bilirubin
26	IL 18		Interleukin 18
27	IRIS	:	International Renal Interest Society
28	IU	:	International unit
29	KIM 1		Kidney Injury Molecule 1

30	**L**	**:**	Liter
31	**MCH**	**:**	Mean Corpuscular Hemoglobin
32	**MCHC**	**:**	Mean Corpuscular Hemoglobin Concentration
33	**MCV**	**:**	Mean Corpuscular Volume
34	**mg**	**:**	Milligram
35	**µg**	**:**	Microgram
36	**µl**	**:**	Microliter
37	**MNC**	**:**	Mononuclear cell
38	**MS**	**:**	Mean Sum of Square
39	**N**	**:**	Neutrophils
40	**NGAL**	**:**	Neutrophil Gelatinase Associated Lipocalin
41	**ng**	**:**	Nanogram
42	**PCV**	**:**	Packed Cell Volume
43	**pg**	**:**	Picogram
44	**RBP**		Renal Binding Protein
45	**RIFLE**	**:**	Risk, Injury, Failure, Loss, End-Stage Kidney Disease
46	**SD**	**:**	Standard Deviation
47	**SE**	**:**	Standard Error
48	**SGOT**	**:**	Serum Glutamic Oxaloacetic Transaminase
49	**SGPT**	**:**	Serum Glutamic Pyruvic Transaminase
50	**sNGAL**	**:**	Serum Neutrophil Gelatinase Associated Lipocalin
51	**SPR**	**:**	Solid Phase Receptacle
52	**SS**	**:**	Sum of Square
53	**TEC**	**:**	Total erythrocyte count
54	**TLC**	**:**	Total leukocyte count
55	**TMB**	**:**	Tetramethybenzidine
56	**TSP or TP**	**:**	Total Serum Protein
57	**uNGAL**		Urine Neutrophil Gelatinase Associated Lipocalin

INTRODUCTION

Kidneys are one of the five vital organs of animal body, involved in entire body homeostasis. The kidneys are two bean-shaped organs found on the left and right sides of the body in vertebrates.The kidneys receive about 20 percent of the heart's blood output and play a vital role in keeping the dog in normal metabolic balance. Each kidney is made up of over a million parallel mass transfer units which receive their common blood supply from renal arteries. These functional units are called nephrons and can be viewed as a sequential arrangement of mass transfer devices (glomerulus, Bowman's capsule, proximal tubule, loop of Henley, distal tubule and collecting duct) for two fluid streams: blood and urine. Kidney function is served by two major mechanisms: ultrafiltration and a combination of passive and active tubular transport of electrolytes and other solutes, together with the water in which they are dissolved, in the complex system provided in the rest of the nephron. They filter the blood in order to make urine, to release and retain water, and to remove waste.

The kidneys regulate the balance of ions known as electrolytes in the blood, along with maintaining acid base homeostasis. They also move waste products out of the blood and into the urine, such as nitrogen containing urea and ammonium. Kidneys also regulate fluid balance and blood pressure. They are also responsible for the reabsorption of water, glucose, and amino acids. It also produce hormones including calcitriol and erythropoietin. The kidneys also make an important enzyme, renin, which affects blood pressure through negative feedback.

Kidney disease is a common problem of dogs, affecting an estimated 10% of canines in their lifetimes. Kidney failure is always life threatening and requires immediate attention. The kidneys are uniquely susceptible to injury due to the large quantity of blood filtered for each cardiac cycle (20-25 % of cardiac output), regional differences in blood supply within the kidney and a high rate of metabolic activity of renal tubular cells. Kidney diseases include any structural or functional change of the kidneys, while kidney failure refers a decline of renal function below a threshold level. Both the kidney disease and failure can occur

acutely or chronically. Although renal disease and renal failure are common in small animal patients, diagnosis and management of renal disease is sometimes challenging due to limitations of early detection of renal injury. There are a number of different kidney diseases and causes that may affect different age groups and have different consequences. Some of important kidney diseases are as follows;

1) Renal dysfunction:- Failure of the filtration function of the kidneys leads to the development of azotemia (an excess of nitrogenous compounds in the blood), which may be classified as prerenal, renal, postrenal, or of mixed origin.

   Prerenal:- Prerenal causes ("pre-renal azotemia") are those that decrease effective blood flow to the kidney and cause a decrease in the glomerular filtration rate (GFR). Both kidneys need to be affected as one kidney is still more than adequate for normal kidney function. Notable causes of prerenal dysfunction include low blood volume (e.g., dehydration), low blood pressure, heart failure (leading to cardiorenal syndrome), liver cirrhosis and local changes to the blood vessels supplying the kidney.

   Intrinsic/ renal:- Intrinsic refers to disease processes which directly damage the kidney itself. It can be due to one or more of the kidney's structures including the glomeruli, kidney tubules, or the interstitium. Common causes of each are glomerulonephritis, acute tubular necrosis(ATN), and acute interstitial nephritis (AIN), respectively.

   Postrenal:- Postrenal refers to kidney injury caused by disease states downstream of the kidney and most often occurs as a consequence of urinary tract obstruction. This may be related to benign prostatic hyperplasia, kidney stones, obstructed urinary catheter, bladder stones, or cancer of the bladder, ureters, or prostate.

2) Glomerular Disease:- Glomerular disease is a well-recognized cause of chronic kidney disease (CKD) in dogs.

3) Tubulointerstitial disease:-Inflammation and damage to the kidney tubules and supporting tissues commonly leads to chronic kidney disease. In many cases there is no identified cause, and thus no option for specific

treatment. This type of kidney disease can only be confirmed by microscopic examination of a kidney biopsy specimen, but biopsies are not usually recommended.

4) Nephrolithiasis:-Some mineral solutes precipitate to form crystals in urine; these crystals may aggregate and grow to macroscopic size, at which time they are known as uroliths (calculi or stones). Uroliths generally contain an organic matrix that is believed to vary minimally among uroliths and that constitutes ~2%–10% of the stone's chemical composition. The remaining 90%–98% of the urolith is composed of minerals that vary depending on the type of urolith. Urolithiasis is a general term referring to stones located anywhere within the urinary tract. Uroliths can develop in the kidney, ureter, bladder, or urethra and are referred to as nephroliths, ureteroliths, urocystoliths, and urethroliths, respectively.

5) Kidney cancer:- also known as renal cancer, is a type of cancer that starts in the cells in the kidney. The two most common types of kidney cancer are renal cell carcinoma (RCC) and transitional cell carcinoma (TCC, also known as urothelial cell carcinoma) of the renal pelvis (Mulders *et al,* 2008).
6) Disorders of micturition:- It result from a dysfunction in the storage or voiding of urine and may beneurogenic or non-neurogenic in origin. Urinary incontinence is the failure of voluntary control ofmicturition, with constant or intermittent unconscious passage of urine. Incontinent animals mayleave a pool of urine where they have been lying or may dribble urine while walking. The coat aroundthe vulva or prepuce may be wet, and peri vulvar or peri preputial dermatitis can result from urine scalding. Failure of urine storage is characterized by inappropriate leakage of urine due to failure of bladderrelaxation, urethral incompetence, anatomic defects, or overflow of stored urine.

Among these diseases renal dysfunction is the main cause of renal failure in dogs.

Renal dysfunction occurs when there is sudden damage to more than 75 percent nephrons. The two main forms are acute kidney injury, which is often reversible with adequate treatment and chronic kidney disease, which is often not reversible.

Acute kidney injuryresults from sudden decline in renal function and is typically associated with ischemia, acute glomerulonephritis, tubular necrosis, or poisoning with “nephrotoxins” (e.g. heavy metals, some aminoglycosidesand excessive loads of free hemoglobin) because of their unique anatomical and physiological features. The large renal blood flow results in increased delivery of blood-borne toxicants to the kidney as compared with other organs.is typically associated with ischemia, acute glomerulonephritis, tubular necrosis, or poisoning with “nephrotoxins” (e.g. heavy metals, some aminoglycosidesand excessive loads of free hemoglobin). Acute tubular necrosis accounts for approximately 76 percent of all causes of acute renal failure (Namita *et al.,* 2005).

The **chronic renal failure** is the most frequently diagnosed renal disorder amongst the renal failure in dogs, especially of ageing dogs. Renal failure is defined as a loss of three-quarters of functioning nephrons and it is associated with various clinical signs. The most common are polyuria and polydipsia due to the loss of concentrating ability. Gastrointestinal complications (inappetence, anorexia, vomiting, diarrhoea, weight loss) are very common and they are usually the first signs. Neurological abnormalities associated with CRFare very common as well. They include dullness, lethargy, tremors, seizures, stupor and coma. Chronic renal failure results from a progressive and irreversible loss of functioning nephrons and the cause being infectious toxic or iatrogenic, vascular traumatic, metabolic, neoplastic, idiopathic and non-immune mediated disorders.is usually caused by chronic glomerulonephritis (of infectious or immune origin), pyelonephritis (ascending infection of the urinary tract) or hypertension (leading to nephrosclerosis).

Nephritis essentially involves the inflammation of the kidneys. It is the responsibility of the kidneys to filter out waste and excess fluid from the body, and when swelling occurs, their ability to filter properly is reduced. When this happens, the body accumulates both excess water and wastes in the blood stream, while blood and protein are lost in the urine. Because nephritis is a

general term used to describe any kidney inflammation, the outcome and severity is entirely dependent on the underlying cause**.** In other cases, nephritis is very serious and may lead to kidney disease such as glomerulonephritis**,** inflammation such as lupus nephritis**,** infections such as pyelonephritis, or even kidney failure in severe cases (Vegad,2005).

Nephrosis is the result of degenerative changes caused by toxins and poisons that target the kidneys or by ischemia.

Renal failure is the most common complication in case of canines now a day. Creatinine and BUN are the traditional markers for detection of renal failure but these markers appear late (approx. after 50-75% damage to nephron) in serum as well as urine. To overcome this problem there are some markers which are used for early detection of renal failure.

There are many kidney biomarkers which are helpful for detection of renal failure such as functional biomarkers (Serum creatinine, Serum cystatin C and urine albumin), Up-regulated proteins (Neutrophil gelatinase associated lipocalin (NGAL), Kidney injury molecule 1 (KIM-1), Liver-type fatty acid–binding protein (LFABP), Interlukin 18 (IL18), β-trace protein (BTP) and Asymmetric dimethylarginine (ADMA)), Low-molecular-weight proteins (Urine cystatin C) and enzymes (N-acetyl-glucosaminidase (NAG), Glutathione-s-transferase (GST), Gamma-glutamyl transpeptidase (GGT), Alanine amino peptidase (AAP) and Lactate dehydrogenase (LDH)).

Among all these biomarkers Neutrophil gelatinase-associated lipocalin (NGAL) is a promising biomarker as it comes in serum or urine within 2 hours of kidney injury where creatinine and BUN increases after 50% to 75% damage of kidney.

Neutrophil gelatinase associated lipocalin (NGAL) is a protein belonging to the lipocalin superfamily initially found in activated neutrophils, in accordance with its role as an innate antibacterial factor. It is a biomarker which is useful for early detection of renal failure as it occurs in serum and urine within 2 hours after kidney injury. NGAL levels predict the future appearance of acute kidney injury after treatments potentially detrimental to the kidney and even the acute worsening of unstable nephropathies (Bolignano *et al*., 2008).

Beta 2 microglobulin is a biomarker which is useful to detect increased levels of serum beta-2 microglobulin which found in patients with kidney disease and are a major constituent of the amyloid fibrils in dialysis related amyloidosis. It also helps in differentiation between glomerular damage and tubular damage as its increase level in serum indicates glomerular damage and in urine indicates tubular damage. Beta 2-Microglobulin is a low molecular weight protein with sequence homology to immunoglobulins. As a portion of the HLA complex this protein is an important cell-surface structure. Under normal conditions beta 2-microglobulin is synthesized and shed by many cells, particularly lymphocytes, and is detectable in the circulation of normal individuals. Because of its small size it is normally filtered readily at the glomerulus and is catabolized by proximal tubular cells of the kidney. Impaired renal function and hyperproduction of beta 2-microglobulin are both associated with increased serum levels (Ahuja M. *et al* 2010).

Considering the importance of renal disorders in canines, the study was undertaken with the following objectives,

1) To study the prevalence of the renal dysfunction in clinical cases of canines.

2) To asses hematobiochemical and urine analysis of canine with renal dysfunction, and

3) To explore the potential of lipocalin and β2 microglobulin biomarkers for early detection of renal dysfunction.

2. REVIEW OF LITERATURE:

2.1 Renal dysfunction:

Macdougall *et al*., (1986) investigated one hundred and eleven dogs with canine chronic renal disease. In 76 cases, clinical details, blood and urine biochemistry, serology and kidney tissue for light and electron microscopy, and imniunohistochemistry were obtained. Forty (52%) had glomerular (GN) and 36 (48%) non-glomerular (NGN) disease. Types of GN identified were (W.H.O. classification): focal glomerulonephritis (GN) (5), diffuse mesangial proliferative GN (8), diffuse endocapillary proliferative GN (2), mesangiocapillaryGN type 1(8), diffuse crescenticGN (I), diffuse sclerosingGN (7), amyloid (6), unclassifiable GN (3). Eight dogs with GN and 13 with NGN had extra-renal lesion. Proteinuria, but not age, breed, sex, serum creatinine or hematuria, discriminated between GN and NGN groups.

Vaden *et al.,* (1997) studied medical records of dogs presented to the hospital for a diagnosis of ARF. The diagnosis of ARF was based on clinical signs, renal imaging findings, and clinicopathologic data and, in most cases, was confirmed by histopathology. 481 dogs presenting to the hospital. Conclusions from this study were as follows: (1) Intact male dogs and nonsporting dogs were more likely to develop ARF and be admitted to the teaching hospital. (2) Dogs with severe azotemia (serum creatinine concentration > 10 mg/dL), hypocalcaemia (<8.6 mg/ dL), and proteinuria were less likely to survive ARF and be discharged from the hospital. (3) Dogs that survived in the hospital for more than 5 days were more likely to recover and be discharged from the hospital.

Sosnar *et al.,* (2003) determined the occurrence of acute renal failure (ARF), chronic renal failure (CRF) and transitional forms, i.e. of ARF developing from chronic renal insufficiency (CRI), in dogs. Total number of admitted patients at the clinic during the period of the study were 935 dogs, the diagnosis of renal failure was determined in 139 (12.7%) of these patients. The essential condition to include apatient in the study was the confirmation of intra-renalazotaemia. An

analysis of monitored parameters was done in each patient in order to differentiate ARF from CRF. Total mortality due to RF was 76.6%. In 45 patients (32.4%) RF was caused by or connected with another disease. In 38 patients (27.3%) only acute symptoms were detected. Chronic changes were demonstrated in 73 patients (52.51%).

Stokes and Bartes (2006) stated that acute renal failure (ARF) is devastating in dogs and cats, with a mortality rate of over 50%.Causes include pre-renal, intrinsic/renal, and post-renal conditions. Infectious disease (leptospirosis, bacterial pyelonephritis, borreliosis) and toxicity (medications, plants, antifreeze) are the most common causes of ARF in dogs and cats. Ureteral obstruction in cats is being recognized more frequently as a post-renal cause of ARF.

Petejova and Martinek (2013) stated that acute kidney injury (AKI) is a common serious complication of severe acute pancreatitis (SAP) and an important marker of morbidity and mortality in critically ill septic patients. AKI due to severe acute pancreatitis can be the result of hypoxemia, release of pancreatic amylase from the injured pancreas with impairment of renal microcirculation, decrease in renal perfusion pressure due to abdominal compartment syndrome, intraabdominal hypertension or hypovolemia.

Oburai *et al.,* (2015) studied 31 CRF dogs, they found clinical and ultrasonographic alterations of chronic renal failure (CRF) in dogs presented. The diagnosis involved clinical observations, hematology, serum biochemical profile, urinalysis and ultrasonography. The respective findings were compared with 10 healthy control dogs. CRF with male predominance especially in 8 to 12 years dogs and in spitz breed was observed. The predominant signs in CRF dogs included anorexia, vomition, dullness, weight loss, oral ulcers, polyuria, and polydipsia, pallor of mucosa, hypertension recumbency and blindness. Blood picture revealed anemia with mild neutrophilic leukocytosis. Serum urea nitrogen, creatinine, sodium and phosphorus levels were significantly elevated whereas total protein and albumin were reduced. Urine had lower specific gravity and contained higher amounts of protein.

2.2 Incidence of renal dysfunction in canines with respect to age, sex and breed:

2.2.1. Incidence of renal dysfunction in dogs with respect to age:

Lucre *et al*., (1980) studiedclinico-pathological findings are described in thirteen young dogs with advanced renal disease. All but three dogs were less than 2 years old. Some had signs of renal dysfunction since birth. Presenting signs were variable but anorexia, lethargy and weight loss were most frequent.

Srinivasan *et al.,* (1993) recorded that out of 50 canine patients diagnosed with primary renal insufficiency, 52 per cent were in the age group of 5 to 10 years, 36 percent were more than 10 years and 12 per cent were less than 5 years age. Chronic renal insufficiency was nearly three times more in males (76.3%) than in females (23.7%). Acute renal insufficiency was also more common in males. It was twice more common in male dogs (66.7%) than in female (33.3%) dogs.

Greco (2001) studied congenital renal diseases at birth and may be determined genetically; familial renal disorders occur in related animals with a higher frequency than would be expected by chance, and frequently are inherited. The most common familial disorders in cats and dogs include renal amyloidosis, renal dysplasia, polycystic kidneys, basement membrane disorders, and tubular dysfunction (Fanconi's syndrome).

Ganti and Rao (2006) stated that interstitial nephritis is more common in older dogs.

Laroute *et al.,* (2005)collected daily urine and assessment of glomerular filtration rate (GFR) and effective renal plasma flow were performed in ten 2-month-old Beagle puppies and ten 6–9 year-old Beagle dogs to identify age-

associated differences in renal function. The main differences in puppies compared to mature dogs were a higher daily urinary volume (+65%), GFR (+87%), free water reabsorption (+159%), a lower daily protein excretion (−88%), and fractional excretion of phosphorus (−35%). Renal function in Beagle puppies, but not mature dogs, was also quite different compared to data published in younger adult dogs.

Mrudula *et al.*, (2005) studied canine nephritis over a period of one year on sixty dogs (60) presented at the Small Animal Clinics, Madras Veterinary College, with clinical signs suggestive of renal insufficiency and 30 dogs with gross nephritis lesions submitted to the department of Veterinary Pathology. The mean age of affected animals was 7.8 ± 0.34 years. Sixty percent of the animals affected were males and 40 % were females. Aged dogs (mean – 7.8 ± 0.34 years) were more susceptible for nephritis. German shepherd (26.66%) and Spitz (21.66%) showed a higher incidence of nephritis. E.coli was the principal organism isolated from urine samples. In this study, out of 56 cases studied histopathologically, majority of the animals showed subacute and chronic type of nephritis. The increased incidence of subacute and chronic type oif nephritis suggested that renal diseases were not diagnosed in the early stages because of the less sensitivity of routinely used screening tests like BUN serum creatinine.

Polzin *et al.,* (2005) studied that estimation of kidney diseases in dogs to be in the range between 0.5-0.7 percent. According to them, mean age of diagnosis of chronic renal failure in dogs was at the age of 6.5 year

Bartlett *et al.,* (2010) concluded that the chronic kidney disease in dogs occurs at the average 9.9 years of age.

Yhee *et al.,* (2010) observed that 5-6 years of age was most common age to be affected with chronic renal failure.

Littman (2011) studied thatrenal failure is arguably the most common organ failure in dogs. The prevalence of glomerular lesions, mostly immune mediated glomerulonephritis (IMGN). was found in 43% to 90% of random dogs.

Increased urine protein creatinine ratio (UPC) as an indicator of glomerular disease, is a negative predictor of outcome. Microalbuminuria is detected in about 25% of all dogs and cats, increasing with age (36% in dogs between 9-1 years 49% in dogs above 12 years).

Segev *et al*., (2012) studied Clinical and pathologic manifestations of RA differ between Chinese Shar-Pei (CSPs) and non-Shar-Pei (NSPs) dogs. Generally renal amyloidosis is recognized most commonly inmiddle-aged to older dogs.In this study, CSPs were significantly younger compared to NSPs, in agreement with previous reports. The relatively early disease presentation in CSPs supports the breed's genetic predisposition for RA. An autosomal recessive trait predisposing CSPs to RA has been suggested previously.

Sharma and Shrestha (2011) conducted a study to evaluate the renal in the dogs. One hundred serum samples were collected from dogs on the basis of common signs and symptoms of renal. The biochemical analysis for kidney function tests was Blood urea nitrogen (BUN) and serum creatinine. In the agewise prevalence, age groups of 5-10 were found to be more vulnerable for renal disorders followed by 10-15 and 0-5 years age group. This study, showed range value of BUN and Creatinine were found to be 7-227 mg/dl and 0.6-41mg/dl respectively.

Kandula and Karlapudi (2014) studied total of 237 dogs were subjected for various diagnostic protocol like, urine analysis, urine enzymology, serum chemistry and ultrasonography for diagnosing renal disorders, 79 cases (33.33%) cases were diagnosed for renal insufficiency associated with various causes, viz., nephritis, cystitis and/urethritis, urolithiasis, pyometra, renal insufficiency associated with cardiovascular and other systemic causes and mixed conditions. Of all the causes nephritis (24.05) was recorded as highest prevalent in dogs. The prevalence was highest (52.63%) among the dogs of above 8 years age and lowest among up to 4 years(10.53%).

Oburai *et al.* (2015) conducted a study in 31 dogs suffering from chronic renal failure. CRF was highest in 8 to 12 years and least in less than 4 years of age. A male predominance over female was observed.

2.2.2. Incidence of renal dysfunction in dogs with respect to sex:

Wilcock and Patterson (1979)found progressive renal disease in 13 related Doberman pinscher dogs had the histological criteria of membranoproliferative glomerulonephritis. Polyuria, polydipsia and weight loss were the usual initial abnormalities and were observed at one year of age or less in seven of I 1 dogs diagnosed antemortem as having renal disease. There was no sex predilection. All dogs were traced to a common male dog no more than four generations previously.

Macdougall *et al*., (1986)stated there was no clear evidence of any predisposition to renal disease related to sex or breed.

Robertson (1986) studied that renal disease is common in dogs. The incidence of significant renal disease increases with age. Many disease processes are subtle and subacute, and so many are not detected until they result in chronic renal failure. The causes of many renal diseases are not known but one must suspect immune-mediated damage in some.

Srinivasan *et al.,* (1993) recorded that chronic renal insufficiency was nearly three times more in males (76.3%) than in females (23.7%). Acute renal insufficiency was also more common in males. It was twice more common in male dogs (66.7%) than in female (33.3%) dogs.

Vaden *et al*., (1997) evaluated retrospectively demographic and clinicopathologic factors that may be associated with the diagnosis and outcome of acute renal failure (ARF) in dogs presented to a large referral hospital. Medical records of dogs presented to the hospital were searched for a diagnosis of

ARF. They found Intact male dogs and nonsporting dogs were more likely to develop ARF and be admitted to the teaching hospital.

Ganti and Rao (2006) stated that interstitial nephritis and uremia is more common in male than female.

Mrudula *et al.,* (2005)studied canine nephritis over a period of one year on sixty dogs (60) presented at the Small Animal Clinics, Madras Veterinary College, with clinical signs suggestive of renal insufficiency and 30 dogs with gross nephritis lesions submitted to the department of Veterinary Pathology. The mean age of affected animals was 7.8 ± 0.34 years. Sixty percent of the animals affected were males and 40 % were females.

Vegad (2005) stated that in dogs, nephritis is 2-3 times more common in male than female.

Sharma and Shrestha (2012) conducted a study to evaluate the renal in the dogs. One hundred serum samples were collected from dogs on the basis of common signs and symptoms of renal. The biochemical analysis for kidney function tests was Blood urea nitrogen (BUN) and serum creatinine. The result showed the higher prevalence of both renal. They found that in sex wise distribution, males were more vulnerable than females.

Kandula and Karlapudi (2014) studied total of 237 dogs were subjected for various diagnostic protocol like, urine analysis, urine enzymology, serum chemistry and ultrasonography for diagnosing renal disorders, 79 cases (33.33%) cases were diagnosed for renal insufficiency associated with various causes, viz., nephritis, cystitis and/urethritis, urolithiasis, pyometra, renal insufficiency associated with cardiovascular and other systemic causes and mixed conditions. Of all the causes nephritis (24.05) was recorded as highest prevalent in dogs. Further renal insufficiency was highly prevalent in female dogs (63.16%).

Oburai *et al.,*(2015) conducted a study in 31 dogs suffering from chronic renal failure. CRF was highest in 8 to 12 years and least in less than 4 years of

age. He observed male dogs were predominately affected by renal failure than female dogs.

2.2.3. Incidence of renal dysfunction in dogs with respect to breed:

Wilcock and Pattessons (1979) studied progressive renal disease in 13 related Doberman pinscher dogs had the histological criteria of membranoproliferative glomerulonephritis. Polyuria, polydipsia and weight loss were the usual initial abnormalities and were observed at one year of age or less in seven of 11 dogs diagnosed antemortem as having renal disease. There was no sex predilection.

Chew *et al.*, (1983) studied renal failure was diagnosed in 22 young Doberman Pinscher dogs. The clinical findings were anorexia, weight loss, vomiting, lethargy, polydipsia, polyuria, and dehydration.

Picut and Lewis (1987[a]) studied ten cases of juvenile renal disease in Doberman Pinschers were examined by light microscopy and 8 of them additionally by electron microscopy. Two distinct basic ultrastructural lesions of the glomerular basement membrane (GBM) were observed. One is characterized by lamellation of the lamina densa with intramembranous focal areas of lucency containing electron-dense particles, the second by diffuse attenuation of the lamina densa with intramembranous and/or subendothelial deposition of matrix entrapping cross-banded fibres (collagen). Based on similar ultrastructural changes in other hereditary nephropathies in man and dogs, a metabolic or biochemical basis for the structural lesions is suspected.

Cook *et al*., (1993) foundatrophicglomerulopathy resulting in chronic renal failure 4 related Rottweilers, each < 1 year old and 4 dogs had severe azotemia and massive protein-losing nephropathy.

Rawdon (2001) studied case of juvenile nephropathy is reported in a 16-week-old Samoyed bitch. Clinical, laboratory and gross postmortem findings

followed by histological analysis of kidney, liver and cerebrum and transmission electron microscopy of renal tissue are described.

Mrudula *et al*., (2005)studied canine nephritis over a period of one year on sixty dogs presented at the Small Animal Clinics, Madras Veterinary College, with clinical signs suggestive of renal insufficiency and 30 dogs with gross nephritis lesions submitted to the department of Veterinary Pathology. They reported that in German shepherd (26.66%) and Spitz a higher incidence of nephritis was present.

Kandula and Karlapudi (2014) studied that the prevalence of renal insufficiency was 33.33% in dogs (n=79). The prevalence rates of various renal disorders including renal tissue pathology associated disorders, cystitis and/or urethritis, urolithiasis, systematic causes associated with renal diseases and mixed infections were 24.04%, 22.79%, 15.19%, 11.39% and 17.72%, respectively. The prevalence was higher in dogs of above 8 years of age (52.63%) as compared to dogs upto 4 years of age (10.53%). The breeds affected were Labrador retriever (21.05%) followed by German shepherd (15.79%) and Boxer, Doberman pinscher, Pomeranian and Pug (5.26%, each).

Oburai *et al.,* (2015) conducted a study in 31 dogs suffering from chronic renal failure. Among the affected breeds, Spitz breed showed highest incidence followed by German shepherd, and least in Doberman pinscher.

2.3 Haematological and biochemical alterations:

Wright *et al*., (1976) studied clinical and morphological analysis of eight cases of chronic glomerulonepshritis is described and a comparison made with eight dogs suffering from severe chronic interstitial nephritis. Clinically and biochemically, the two diseases were virtually indistinguishable, both resulting in uraemia. A possible distinguishing feature of chronic interstitial nephritis was the anaemia which was absent from chronic glomerulonephritis cases.

Sperschneider (1977) stated that during a chronic renal insufficiency an anaemia develops. The pathogenetic points of view are very diverse and include disturbances of the formation of erythrocytes and a shortened life span of the erythrocytes.

Picut (1985) reported that there was decrease in the values of PCV, RBC Hb and normocytic normochromic anemia in hereditary kidney disease in dogs. He further stated that there was two or threefold elevation in the levels of BUN and creatininelevels too.

Macdougall *et al*., (1986) found one hundred and eleven dogs with canine chronic renal disease, presented to 24 veterinary practices in East Anglia and the West Midlands (geographical area 8,600 squaremiles) were identified. More than 20 different breeds were represented.In 76 cases, clinical details, blood and urine biochemistry were obtained. Forty had glomerular (GN) and 36 non-glomerular (NGN) disease. They found that level of creatinine did not differ significantly between the two groups.

Cowgill (1992) while studying the chronic renal failure cases in dogs reported that the dehydration as a result of renal insufficiency may lead to reduction in haematocrit value by an average of 0.15 percent points per day.

Oishi *et al*., (1993) evaluating the erythropoietin (EPO) production response to phlebotomic stimulation in the 1/2- and 1/4-kidney dogs surgically prepared. The results showed that the reduction in functional renal tissue caused a decrease in EPO production, which led to the delay in recovery from anemia. In the anemic progress stage, the plasma EPO level showed a transition proportional to the quantity of functional renal tissue immediately after the operation for tissue reduction. The 1/2-kidney dog group still kept such proportional relation even in the recovery stage. Thus, the half of the normal renal tissue was considered sufficiently contributory to EPO production needed to maintain homeostasis of red blood cell production. However, the 1/4-kidney dog group precipitously decreased in plasma EPO level in the recovery stage and fell into an extremely unfavorable anemia. This indicated that homeostatic

maintenance in erythropoiesis would be impossible more below a quarter of normal renal tissue. These findings disclosed that reduction in functional renal tissue quantity would sensitively influence homeostatic maintenance of red blood cell production through the decrease in EPO production, even if it does not affect renal function concerned with urine production.

Oishi *et al*., (1995) determined erythropoietin (EPO) levels in plasma from 124 clinically anemic dogs were determined by in vivo bioassay. In 81 anemic dogs with normal renal function, the concentration of plasma EPO showed a close correlation with the hemoglobin concentration. The plasma EPO level was obviously decreased in 43 anemic dogs with renal failure. Of these dogs with renal failure, 17 showed no detectable plasma EPO and resulted in the death of these dogs. In the remaining 26 dogs having detectable plasma EPO, the plasma concentration rate of EPO related to blood urea nitrogen and serum creatinine values.

Miyamoto *et al.,* (1997) reported a case of six-month-old male Golden retriever with a three-month history of polyuria and polydipsia. Haematological examinations revealed nonregenerative anaemia, azotaemia, high serum creatinine level, hypercalcemia, hypophosphatemia, hypercholesterolemia, hyperamylasemia, and low level of total serum protein.

Adin and Cowgill (2000) studied 36 cases of dogs suffering from leptospirosis and reported acute renal failure in all cases. The average BUN concentration was 122 ± 71 mg/dL whereas the average Serum creatinine concentration was 7.5 ± 5.0 mg/dL in cases of leptospirosis resulting in acute renal failure.

Benjamin (1985) cited that elevated serum levels of BUN and creatinine indicates kidney damage.

DiBartola (2005) stated that serum creatinine is considered to be a more reliable parameter than urea for evaluating renal function.

Robertson and Seguin (2008) stated anemia occurs in up to 50% of dogs with chronic renal failure and is often normocytic, normochromic and non-

regenerative reduced production of or response to erythropoietin. Neutrophilic leucocytosis can be seen with a variety of inflammatory lesions of the renal system. Chronic renal failure can be associated with lymphopenia, which reflects the effects of endogenous glucocorticoids or stress of chronic disease. Mild mature neutrophilia is commonly seen associated with this glucocorticoid effect, too. Increases in urea in renal failure are caused by impaired ability to excrete proteinaceouscatabolites because of marked reduction in glomerular filtration rate (GFR). Increases in creatinine are also a result of decreased renal excretion. Hypoalbuminemia can occur secondary to renal loss in PLN. Hypoalbuminemia may also be seen with renal inflammatory disease (albumin is a negative acute phase reactant). Hyperalbuminemia can occur secondary to hemoconcentration from dehydration in any type of renal disease.

Koyner *et al*., (2010) studied diagnostic and prognostic utility of novel and traditional AKI biomarkers was evaluated during a prospective study of 123 adults undergoing cardiac surgery. They found that AKI patient had higher baseline serum creatinine.

Polzin (2011) noted hematobiochemical changes in chronic kidney disease. The haematological data revealed normocytic normochromic, hyperproliferativeanemia as the renal function deteriorates in dogs with chronic kidney disease. The anemia in chronic renal disease is due to inadequate renal production of the hormone erythropoietin which is essential for erythrocyte production. The ulcerations due to uremia leads to chronic low-grade gastrointestinal blood loss resulting in moderate to severe anemia, decreased red blood cells life span in uremic environment and chronic inflammation.

Sharma and Shrestha (2011) evaluated the renal disorder in the dogs. One hundred serum samples were collected from one hundred dogs showing the common signs and symptoms of renal. The result showed the higher prevalence of renal disorder. There were increase in BUN and creatinine level.

Bradea *et al*., (2013)studied dogs with progressive chronic renal failure register hematological alteration of different intensities reflected on the complete blood count parameters. Dogs with CKD typically present normochromic,

normocytic, nonregenerative anemia, induced by conjunctive substitution phenomenon in renal parenchyma level with reduced erythropoietin secretion in this level. 12 dogs with CKD were included in this study to evaluate changes in hematological parameters. This study correlated hematological parameters (red blood cells and white blood cells) with parameters that reveal and characterize the level of renal functional alteration in CKD (blood urea nitrogen, creatinine, phosphorus, sodium and potassium). In 10 out of 12 individuals, an alteration in hematological profile elements by decreased levels (hypochromeanemia)with hematocrit values that varied between 15.2% and 33.5% and a decrease in hemoglobin levels between 6.4 and 11.7 g/dl. Platelets number was near the upper limit and in severe cases, in 2 dogs (16.66%) were over 682 K/µL. White blood cells series registered inconstant and uncharacteristic modifications correlated mainly with etiopathogenetic inductor context such as lymphopenia in 4 dogs (33.33%) varying from 24.58 and 39.6 K/µL, granulocytes increased levels in 3 out of 12 dogs (25%) varying between 14.7 -16.2 K/µL.

Kandula and Karlapudi (2015) studied renal insufficiency induced haematological and serum biochemical alterations in affected dogs. The study was conducted on 79 dogs presented at the clinic showing clinical signs of renal insufficiency and ten healthy dogs were included in the study that served as the control. The study revealed that the haematological parameters, *viz.*, TEC, Hb and PCV were significantly lower, and the TLC was significantly higher in dogs with renal insufficiency, compared to the normal dogs. The neutrophils were significantly higher, while lymphocytes and eosinophils were significantly lower, compared to the normal dogs, indicating leucocytosis with Neutrophilia, Lymphopenia, and Eosinopenia. The serum biochemical parameters, *viz.*, Blood Urea Nitrogen and Creatinine were significantly higher in dogs with renal insufficiency, compared to the normal dogs, while Total Proteins, Albumins, were significantly lower than the normal dogs.

Oburai *et al.,* (2015) conducted a study in 31 dogs suffering from chronic renal failure. The blood picture revealed anemia with mild neutrophilic leucocytosis. Serum urea nitrogen, creatinine, sodium and phosphorus levels were significantly elevated whereas total protein and albumin were reduced. CRF dogs had a significantly ($P<0.05$) lower erythrocyte count and an associated

decrease in packed cell volume and haemoglobin concentration compared to control dogs.

2.4 Urinalysis:

Various studies have indicated a positive co-relationship between persistent proteinuria and renal insufficiency and mortality. Therefore it is essential to give proper attention to urine analysis.

Brown *et al*., (1985) studied Gentamicin-associated acute renal failure was diagnosed in 10 dogs. The disease was characterized by a poor prognosis and lengthy hospitalization. Hypoalbuminemia, disorders of potassium homeostasis, proteinuria, hematuria, and cylindruria were common during therapy for renal failure. Fever and dehydration were the most commonly identified potential predisposing factors.

Picut and Lewis (1987[b]) studied hereditary nephropathies in dogs represent multiple complex clinicopathological entities which cause renal failure in juvenile, adolescent, and young adult dogs. He concluded that the specific gravity of urine was decreased to 1.008 to 1.010 g/ml and found that damaged kidney tubules were not able to concentrate urine leads to dilution of urine.

Robertson and Seguin (2008) stated that patients in renal failure can have isosthenuric urine (specific gravity [SG] 1.008-1.012). Cats and dogs with early renal insufficiency may have minimal ability to concentrate urine. A SG of less than 1.035 in a dehydrated feline patient or less than 1.025 in a dehydrated canine patient is considered suspicious for decreased renal function. Concentrating ability generally decreases before the development of azotemia. Patients with PLN may maintain some concentrating ability before the development of secondary tubular damage. An active sediment (bacteriuria, white blood cells, red blood cells or casts) may suggest infection. An attempt should be made to localize the infection to the upper or lower urinary tract because this may affect therapy. Casts are cylindric molds of the renal tubules and are composed of aggregated proteins or cells. The presence of casts in the

urine sediment localizes activity to the kidney itself. Occasional hyaline and rarely seen fine granular casts per low power field may be considered normal if there are no other findings associated with renal disease. Cellular, coarse granular and waxy casts are always pathologic. The UPC offers a technique for quantitative measurement of proteinuria in dogs and cats. The UPC can be used to screen for early renal disease.

Harley and Langston (2012) described proteinuria is defined as the presence of protein in the urine. Normally, circulating serum proteins are blocked by the glomerulus due to size and/or charge. Any small proteins that pass through a healthy glomerulus are reabsorbed by the renal tubules or broken down by renal tubular epithelial cells. Persistent proteinuria, in the absence of lower urinary tract disease or reproductive tract disease, is usually an indication of renal damage or dysfunction. Less commonly persistent proteinuria can be caused by increased circulating levels of low molecular weight proteins. This article reviews mechanisms of proteinuria in dogs and cats and discusses the importance of screening for and ultimately treating proteinuria.

Oburai *et al.,* (2015) in their study in 31 dogs suffering from chronic renal failure observed that urine had lower specific gravity and contained higher amounts of protein.

2.5 Estimation of biomarkers:

2.5.1 Lipocalin estimation:

Dent *et al*., (2007) studied acute kidney injury (AKI) is a frequent complication of cardiopulmonary bypass (CPB). First, in a cross-sectional pilot study including 40 plasma samples (NGAL range 60 to 730 ng/ml) and 12 calibration standards (NGAL range 0 to 1,925 ng/ml), NGAL measurements by enzyme-linked immunosorbent assay and by Triage NGAL Device were highly correlated (r = 0.94). Second, in a subsequent

prospective uncontrolled cohort study, 120 children undergoing CPB were enrolled. Plasma was collected at baseline and at frequent intervals for 24 hours after CPB, and analyzed for NGAL using the Triage(R) NGAL device. AKI developed in 45 patients (37%), but the diagnosis using serum creatinine was delayed by 2 to 3 days after CPB. In contrast, mean plasma NGAL levels increased threefold within 2 hours of CPB and remained significantly elevated for the duration of the study. For the 2-hour plasma NGAL measurement, the area under the curve was 0.96, sensitivity was 0.84, and specificity was 0.94 for prediction of AKI using a cut-off value of 150 ng/ml. The 2 hour postoperative plasma NGAL levels strongly correlated with change in creatinine ($r = 0.46$, $P < 0.001$), duration of AKI ($r = 0.57$, $P < 0.001$), and length of hospital stay ($r = 0.44$, $P < 0.001$). Plasma NGAL is an early predictive biomarker of AKI, morbidity, and mortality after pediatric CPB.

Bolignano *et al*., (2008) stated that neutrophil gelatinase-associated lipocalin (NGAL) is a protein belonging to the lipocalin superfamily initially found in activated neutrophils, in accordance with its role as an innate antibacterial factor. However, it subsequently was shown that many other types of cells, including in the kidney tubule, may produce NGAL in response to various injuries. The increase in NGAL production and release from tubular cells after harmful stimuli of various kinds may have self-defensive intent based on the activation of specific iron-dependent pathways, which in all probability also represent the mechanism through which NGAL promotes kidney growth and differentiation. NGAL levels predict the future appearance of acute kidney injury after treatments potentially detrimental to the kidney and even the acute worsening of unstable nephropathies. Furthermore, recent evidence also suggests that NGAL somehow may be involved in the pathophysiological process of chronic renal diseases, such as polycystic kidney disease and glomerulonephritis. NGAL levels clearly correlate with severity of renal impairment, probably expressing the degree of active damage underlying the chronic condition. For all these reasons, NGAL may become one of the most promising next-generation biomarkers in clinical nephrology and beyond.

Devarajan (2008) studied the incidence of both acute kidney injury (AKI, previously referred to as acute renal failure) and chronic kidney disease (CKD). The evidence for the role of NGAL measurements in a variety of clinical situations leading to AKI (cardiac surgery, kidney transplantation, contrast nephropathy, haemolytic uraemic syndrome and in the intensive care setting) or to CKD (lupus nephritis, glomerulonephritides, obstruction, dysplasia, polycystic kidney disease, IgA nephropathy) is explored. The emerging utility of standardized clinical platforms for reliable measurement of NGAL in plasma and urine will help to improve prognosis.

Nguyen and Devarajan (2008) studied acute kidney injury (AKI), previously referred to as acute renal failure (ARF), represents a persistent problem in clinical medicine. These include a plasma panel [neutrophil gelatinase-associated lipocalin (NGAL). In renal failure it occurs early in plasma.

Yilmaz *et al.,* (2009) studied sixty patients with symptomatic UTI and 29 healthy controls and measured their urine NGAL by enzyme-linked immunosorbent assay. Mean uNGAL level was significantly higher in the UTI group than in the controls (91.02 ng/ml vs 14.29 ng/ml, p = 0.0001) and using a cutoff 20 ng/ml for uNGAL for diagnosis of UTI, sensitivity, and specificity were 97% and 76%, respectively. Mean uNGAL/creatinine ratio (uNGAL/Cr) was also significantly higher in the UTI group [201.81 ng/mg creatinine (Cr) vs 18.08 ng/mg Cr; p = 0.0001], and using a cut off 30 ng/mg Cr for uNGAL/Cr for diagnosis of UTI, sensitivity and specificity were 98% and 76%, respectively. They have concluded that both uNGAL and uNGAL/Cr can be used as a novel, sensitive marker for early prediction of UTI in the absence of acute kidney injury and chronic kidney disease, and the optimal cutoff value for prediction of UTI is lower than the values determined for acute kidney injury.

Cruz *et al.,* (2010) performed this study to estimate the diagnostic accuracy of plasma NGAL for early detection of AKI and need for renal replacement therapy (RRT) in an adult intensive care unit (ICU). 301 patients were included in the analysis. Serial blood samples were analyzed for plasma NGAL using a standardized clinical platform. Plasma NGAL was a good diagnostic marker for AKI development within the next 48 h and for RRT use.

Plasma NGAL is a useful early marker for AKI in a heterogeneous adult ICU population, in which the timing of renal insult is largely unknown. It allows the diagnosis of AKI up to 48 h prior to a clinical diagnosis based on AKI consensus definitions. Additionally, it predicts need for RRT and correlates with AKI severity.

De Geus *et al.,* (2011) studied the ability of plasma and urine NGAL to predict severe AKI in adult critically ill patients. Samples were analyzed by Triage immunoassay for NGAL expression. The primary outcome measure was occurrence of AKI based on Risk-Injury-Failure (RIFLE) classification during the first week of ICU stay. A total of 171 (27%) patients developed AKI. Of these 67, 48, and 56 were classified as RIFLE R, I, and F, respectively. The areas under the receiver operating characteristic curves for plasma and urine NGAL were for RIFLE R (0.77 ± 0.05 and 0.80 ± 0.04, respectively), RIFLE I (0.80 ± 0.06 and 0.85 ± 0.04, respectively), and RIFLE F (0.86 ± 0.06 and 0.88 ± 0.04, respectively) and comparable with those of admission estimated glomerular filtration rate (eGFR) (0.84 ± 0.04, 0.87 ± 0.04, and 0.92 ± 0.04, respectively).

Lee *et al*., (2011) studied retrospective case-series to evaluated the prognosis of 853 dogs with acute kidney injury (AKI) based on the RIFLE (Risk, Injury, Failure, Loss and End-stage renal failure) criteria, derived from human medicine. The 30-day mortality of dogs with AKI in each class was found to be 23.8 per cent (40 of 168) dogs for Risk, 41.0 per cent (107 of 261) dogs for Injury and 78.5 per cent (333 of 424) dogs for Failure. The mortality of dogs in either the Injury or Failure class was significantly higher than that of dogs in the Risk class ($P<0.05$). The longest median survival time was observed in the Risk class (nine days) and the shortest median survival time was observed in the Failure class (three days). Using a multiple logistic regression model, a new score that simultaneously considered RIFLE class, diarrhoea status and serum phosphorus level was calculated to predict prognosis.

Adiyanti and Loho (2012) stated that the kidney has a remarkable capacity to withstand insults for an extended period of time. The Acute Kidney Injury Network (AKIN) defined Acute Kidney Injury (AKI) as "functional and structural disorder or signs of renal damage including any defect from blood and urine test, or tissue imaging that is less than 3 months". RIFLE (Risk, Injury,

Failure, Loss, End-Stage Kidney Disease) criteria is the most frequently used system. Ideal biomarker for AKI should be affordable, quick and measurable, precise and accurate, with prognostic ability to define severity of renal dysfunction, specific for renal, increase in the early stage dysfunction, with high sensitivity and specificity. Efforts to detect AKI in the earlier stage has resulted in some promising biomarkers such as KIM-1, NGAL, IL-18, Clusterin, etc. Cystatin C is a biomarker for glomerular filtration function, while beta2-microglobulin, 1-microglobulin, NAG, RBP, IL-18, NGAL, Netrin-1, KIM-1, Clusterin, Sodium Hydrogen Exchanger Isoform and Fetuin A are biomarkers for tubular reabsorption function.

Fagundes *et al.,* (2012) studied impairment of kidney function in two-hundred and forty-one patients with cirrhosis, 72 without ascites, 85 with ascites, and 84 with impaired kidney function. Urinary levels of NGAL were measured by ELISA. Patients with impaired kidney function had higher urinary NGAL levels compared to patients with and without ascites. Patients with urinary tract infection (n = 25) had higher uNGAL values than non-infected patients. Patients with acute tubular necrosis (ATN) had uNGAL levels markedly higher (417 µg/g creatinine (239–2242) median and IQ range) compared to those of patients with pre-renal azotemia due to volume depletion 30 (20–59), chronic kidney disease (CKD) 82 (34–152), and hepatorenal syndrome (HRS) 76 (43–263) µg/g creatinine ($p < 0.001$ for all).

Lee *et al.,* (2012) studied lipocalin for the early prediction of canine acute kidney injury (AKI). 39 dogs at different time points after surgery were selected for study and NGAL was measured in serum and urine with ELISA. At 12 h after surgery, compared to the group without AKI (12 dogs), the NGAL level in the urine of seven dogs with AKI was significantly increased (median 178.4 pg/mL vs. 88.0 pg/mL), and this difference was sustained to 72 hours. As the increase in urine NGAL occurred much earlier than the increase in serum creatinine, urine NGAL seems to be able to serve as a sensitive and specific biomarker for the prediction of AKI in dogs.

Schinstock *et al.,* (2012) examined random and 24 h urine samples obtained from 125 normal volunteers foranalytic validation of aurinaryenzyme-linked immune sorbent assay for NGAL. NGAL was stable in urine for 7 days when ambient, 4°C or frozen (−20 or −70°C). The assay was linear between 0.24 and10000 ng/mL with a limit of quantitation of 0.24 ng/mL. Intra- and inter-assay precision were excellent (coefficient of variation <5%); however, urinary white blood cells were associated with increased NGAL levels. The 95th percentile reference value for NGAL in females is ≤65.0 and ≤23.4 ng/mL in males. Urinary NGAL levels increased with AKI stage but had only fair sensitivity (65%) and specificity (65%) to differentiate no AKI versus Stages 1, 2 or 3 (area under the curve 0.70). Urinalysis with microscopy was very specific (91%) but not verysensitive (22%) with an area under the curve of 0.57. Increased urinary NGAL was associated with AKI by the AKIN criteria; however, the sensitivity and specificity were only fair, in part because patients with prerenal causes are not excluded by AKIN criteria.

Ahn and Hyun (2013) conducted study on sixty-two dogs with CKD and eight normal healthy dogs were enrolled in this study. All dogs were also physically examined, including complete blood cell counts, routine biochemical panels and urinalysis to screen for the presence of other concurrent diseases such as, chronic inflammatory diseases and other systemic diseases. The dogs with CKD were grouped based on the international renal interest Society (IRIS) stage classification, and selected animals at stages I-IV. The supernatants were stored at −80°C or dry ice for shipping. The serum NGAL levels were measured using a commercial ELISA-based kit (BioPorto, Denmark) according to the manufacturer's recommendations. The median serum NGAL concentration in this study population was 12.89 (range 5.03–19.35) pg/ml in the control group, 43.95 (range 22.19–157.36) pg/ml in the IRISi group, 34.89 (range 23.07–108.54) pg/ml in the IRIS ii group, 53.35 (range 20–200) pg/ml in the IRIS iii group and 76.04 (range 68.69–200) pg/ml in the IRIS V group. The median serum NGAL concentration in control dogs was approximately 4–10 times lower than dogs with CKD.

Cobrin (2013) stated that canine chronic kidney disease (CKD) is estimated to have a prevalence of 0.57%. Neutrophil gelatinase-associated

lipocalin (NGAL), a protein detectable in blood and urine that increases secondary to renal dysfunction.She studied 42 dogs free of urinary tract disease (normal history, physical examination, clinicopathologic results, and blood pressure measurement), 11 dogs with CKD, 21 dogs with lymphosarcoma, 12 dogs with carcinomas, and 16 dogs with induced endotoxemia. Serum and urine NGAL concentrations were measured using a commercially available canine-specific ELISA kit. She found that Serum (sNGAL (ng/ml) 7.77 (2.88-35.3) in normal dogs and 24.79 (2.79-61.3) in dogs with CKD) and urine NGAL (uNGAL (ng/ml) 0.55 (0.12-12.90) in normal dogs and 26 (0.77-82.14) in dogs with CKD)levels were elevated in dogs with CKD compared to that of normal dogs.

Daure *et al.,* (2013) stated that neutrophil gelatinase-associated lipocalin (NGAL) is a promising biomarker in humans and dogs with kidney disease. Urine culture and measurement of uNGAL level were performed in 80 non-azotemic dogs suspected of UTI and 19 healthy dogs. Dogs were divided in three groups: 19 healthy dogs, 25 dogs with positive culture and 55 dogs suspected of UTI but with negative culture. uNGAL and uNGAL/Creatinine was significantly higher ($P < 0.0001$) in dogs with UTI (14.22 ng/mL;19.74 µg/g) compared to Healthy (0.24 ng/mL;0.11 µg/g) and Negative (1.13 ng/mL;1.28 µg/g) dogs. AuNGAL value <3.38 ng/mL had a negative predictive value for UTI of 87%. Presence of UTI has to be considered when uNGAL is used to detect kidney disease.

Moon *et al.*, (2013) studied urinary biomarker of acute kidney injury (AKI). Urinary biomarker, neutrophil gelatinase associated lipocalin (NGAL) was measured every 2 days for 8 days in 66 patients with AKI. At day 0, he found that there were no significant differences in plasma creatinine and BUN between AKI patients in the recovery (n = 33) and non-recovery (n = 33) groups. Plasma creatinine concentrations were significantly lower in the recovery group (3.0 ± 2.0 mg/dL) than in the non-recovery group (5.4 ± 1.9 mg/dL) on day 4 after AKI diagnosis ($P < 0.001$). In contrast, there were significant differences in urine NGAL between the two groups starting on day 0 (297.2 ± 201.4 vs 407.6 ± 190.4 ng/mL, $P = 0.025$) through the end of the study (123.7 ± 119.0 vs 434.3 ± 121.5 ng/mL, $P < 0.001$). This observations state that urine NGAL can be a highly

versatile marker for early detection of the recovery phase in established AKI patients.

Segev *et al.,* (2013) studied urine neutrophil gelatinase-associated lipocalin (NGAL)/creatinine in ninety-four dogs. Dogs were classified as follows: (1) healthy dogs, (2) dogs with lower urinary tract disorders, (3) dogs with chronic kidney disease (CKD), (4) dogs with azotemic International Renal Interest Society (IRIS) AKI Grades II–V, and (5) dogs with IRIS AKI Grade I (nonazotemic). Urinary NGAL was quantitated in each dog using an ELISA assay and concentrations were expressed as a ratio to urinary creatinine concentration from the same specimen, and designated the urinary NGAL/creatinine ratio (UNCR). Both the azotemic and nonazotemic AKI groups had higher UNCR when compared with all other groups ($P < .001$ for all pairs). There was a statistically significant difference in UNCR between dogs diagnosed with CKD compared with dogs with lower urinary tract diseases ($P = .005$) as well as between dogs with CKD and healthy dogs ($P = .001$).

Hsu *et al.,* (2014[a]) studied serum and urine NGAL in dogs with renal disease (57) and control dogs without any disease (12). NGAL was measured by ELISA system. Demographic, hematologic, and serum biochemical data were recorded. Survival attributable to AKI and CKD was evaluated at 30 days and 90 days, respectively. Serum and urine NGAL concentrations in azotemic dogs were significantly higher than in nonazotemic dogs and were highly correlated with serum creatinine concentration ($P < .05$). Among CKD dogs, death was associated with significantly higher sNGAL and uNGAL concentrations compared with survivors. The best cutoff point for sNGAL was 50.6 ng/mL, which gave a sensitivity and a specificity of 76.9 and 100%, respectively. They observed dogs that had higher concentrations of sNGAL survived for a significantly shorter time. sNGAL is a useful prognostic marker when evaluating dogs with CKD.

Hsu *et al.,* (2014[b]) examined eighty-one urine samples from dogs with different forms of renal disease (41), pyuria (19) and a number of non-renal related diseases (10), as well as healthy dogs (11).uNGAL concentrations was measured by ELISA. The uNGAL concentrations of dogs with pyuria (median: 15.35 ng/mL) were significantly higher than those of the healthy control animals

(median: 3.92 ng/mL) ($p < 0.01$), but lower than those of dogs with renal diseases (median: 23.77 ng/mL).

Martensson and Bellomo (2014) studied that neutrophil gelatinase-associated lipocalin (NGAL) has been considered the most promising biomarker of acute kidney injury (AKI). Systemic inflammation, commonly seen in critically ill patients, and several comorbidities contribute to the release of NGAL from haematopoietic and non-haematopoietic cells. They concluded that adding NGAL to the clinical evaluation of critically ill patients with or at risk of AKI will give good prognosis.

Steinbach *et al*., (2014) studied dog's plasma NGAL concentration and urine NGAL-to-creatinine ratio (UNCR) in 18 healthy control dogs, 17 dogs with CKD, and 48 dogs with AKI. Plasma and urine NGAL concentrations were measured with a commercially available canine NGAL Elisa Kit (Bioporto Diagnostic) and UNCR was calculated. Median (range) NGAL plasma concentration in healthy dogs, dogs with CKD, and AKI were 10.7 ng/mL (2.5–21.2), 22.0 ng/mL (7.7–62.3), and 48.3 ng/mL (5.7–469.0), respectively. UNCR was 2×10^{-8} (0–46), $1{,}424 \times 10^{-8}$ (385–18,347), and $2{,}366 \times 10^{-8}$ (36–994,669), respectively. Plasma NGAL concentration was significantly higher in dogs with AKI compared with dogs with CKD ($P = .027$).Plasma NGAL could be helpful to differentiate AKI from CKD in dogs with renal azotemia.

Cortellini *et al*., (2015) examined fifteen dogs with sepsis requiring laparotomy (study dogs) and 10 dogs undergoing surgery for intervertebral disc disease (control dogs) to determine whether NGAL increases in dogs with sepsis undergoing emergency laparotomy and whether it is correlated with development of AKI and survival. Serum neutrophil gelatinase-associated lipocalin (sNGAL), urinary NGAL normalized to urinary creatinine concentration (UNCR), and serum creatinine concentration were measured at 4 time points (admission, after anesthesia, and 24 and 48 hours postsurgery). Development of AKI (increase in serum creatinine concentration of 0.3 mg/dL) and in-hospital mortality were recorded.Serum NGAL and UNCR were significantly higher in study dogs across all time points ($P = .007$ and $P < .001$, respectively) compared with controls.

Serum neutrophil gelatinase-associated lipocalin and UNCR are increased in dogs with sepsis requiring emergency laparotomy.

2.5.2 Beta 2 microglobulin:-

Hall and Vasiljevic (1973) studied simple immunodiffusion test for β2-microglobulin which will measure concentrations above 16 µg/ml. This concentration is readily achieved in the urine in conditions associated with primary renal tubular disorders but not in the normal urine or that from patients with glomerular diseases. β2-Microglobulin proteinuria can be detected in untreated urine from individuals whose urines are negative for protein by dipstick and sulfosalicylic acid screening methods. The data show that this method is highly selective and more sensitive than the other methods tested in screening populations for the presence of tubular proteinuria.

Bernier (1980) Stated that beta 2-Microglobulin is a low molecular weight protein with sequence homology to immunoglobulins. As a portion of the HLA complex this protein is an important cell-surface structure. Under normal conditions beta 2-microglobulin is synthesized and shed by many cells, particularly lymphocytes, and is detectable in the circulation of normal individuals. Because of its small size it is normally filtered readily at the glomerulus and is catabolized by proximal tubular cells of the kidney. Impaired renal function and hyperproduction of beta 2-microglobulin are both associated with increased serum levels. A function for beta 2-microglobulin as a modulator of lymphocyte surface and as a potential regulator of the immune system is proposed.

Karlsson *et al.,* (1980) stated that the production of beta 2-microglobulin in normal subjects is quite constant, about 0.13 mg/h kg. The catabolism almost exclusively through renal elimination. The protein readily passes the glomerular membrane; subsequently more than 99.9% of the filtered beta 2-microglobulin is reabsorbed and degraded in the proximal tubules, only about 5 micrograms/h of the protein appearing in the final urine. Proximal tubular dysfunction leads to an increased urinary concentration. The serum level of beta 2-microglobulin is

determined by the glomerular filtration rate and the rate of synthesis. Increased production, with raised serum levels, is sometimes observed in malignancy--mainly at an advanced state, in conditions with neoplastic proliferation of lymphoid B-cells or in inflammatory disorders connected with an activation of the lymphopoetic system. In body fluids, other than plasma and urine, the content of beta 2-microglobulin seems to often reflect a local production. In this review a presentation of the current status and usefulness of beta 2-microglobulin measurements in clinical medicine is included.

Weise *et al*., (1981) stated that urinary excretion of the low molecular weight protein beta 2-microglobulin is very sensitive parameters for proximal tubular lesions. In patients with preeclampsia the renal excretion of beta 2-microglobulin allows to differentiate between a primary preeclampsia and a preeclampsia superimposed upon chronic pyelonephritis. In the first group the increase is 3 to 4 fold and in the second group up to 300 fold. In patients with kidney transplantation the urinary excretion of beta 2-microglobulin is several times higher than in normals. In case of a rejection episode a further increase of these proteins occur in more than 80% several days before clinical symptoms are present. The application of analgetics (paracetamol, acetylsalicylic acid) in healthy individuals in therapeutical dosages on 3 consecutive days does not show any tubular alteration by the measurement of urinary beta 2-microglobulin. Aminoglycosides (tobramycin, UK 18,892) lead to a cumulative increase of the renal excretion of beta 2-microglobulin and AAP while cephalosporins induce an increase of total proteins in the final urine under the same conditions.

Portman *et al.,* (1986) studied fractional excretion (FE) of beta 2 microglobulin (B2M) in children with glomerular (N 114), tubular (N 50) or other (N = 18) renal diseases. FE-B2M (normal 0.36%) was significantly (P0.001) lower in glomerular diseases (mean 0.104%) than in tubular lesions (mean 4.27%). Unexpectedly, several patients with glomerular disease were found to have increased values for FE-B2M. To determine whether this was due to a tubular component in a primary glomerular disease process, FE-B2M was measured in 30 children with various glomerulopathies who underwent renal biopsy. Thirteen of these patients had tubulo-interstitial lesions in addition to their glomerular disease. FE-B2M in these patients averaged 3.76% (range 0.14 to 44.6%); only

two results were normal. Mean FE-B2M in the 17 patients without biopsy evidence of tubulo-interstitial disease was 0.063% (range 0.02 to 0.34%); all values were in the normal range.

Ahuja *et al.,* (2010) stated that increased levels of serum beta-2 microglobulin are found in patients with kidney disease and are a major constituent of the amyloid fibrils in dialysis related amyloidosis

Zeng *et al.,* (2014) studied after filtration through glomeruli, beta 2-microglobulin is reabsorbed in proximal tubules. Increased urinary beta 2-microglobulin indicates proximal tubule injury and measurement of beta 2-microglobulin in urine is useful to determine the source of renal injury. This study was designed to evaluate the correlation of urinary beta 2-microglobulin concentration and KIM-1 expression as evidence of proximal tubule injury. Diagnoses included glomerular and tubule-interstitial disease. Linear regression analysis was applied to correlate the values of urinary beta 2-microglobulin and KIM-1 staining scores and $P < 0.05$ was considered statistically significant. Thirty patients had elevated urinary beta 2-microglobulin. KIM-1 staining was positive in 35 kidney biopsies. There was a significant correlation between urinary beta 2-microglobulin and KIM-1 staining ($P < 0.05$). Sensitivity was 86.6%, specificity was 43.7%, positive predictive value was 74.2%, and negative predictive value was 63.6%. Increased urinary beta 2-microglobulin is significantly correlated with KIM-1 staining in injured proximal tubules. Measurement of urine beta 2-microglobulin is a sensitive assay for proximal tubule injury.

2.6 Gross lesions of renal disorders in dogs:

Ganti and Rao (2006) observed various types of gross lesions in kidney. In pyaemic nephritis**,** tiny abscesses were noticed on the cortical surface (same age and same size).In pyelonephritiskidneys were enlarged; grey spots were noticed on the surface. Pelvis was dilated with pus. In interstitial nephritis in the acute type, the kidney may be of normal size or slightly enlarged. Capsule strips off easily. In subacute and chronic types of interstitial nephritis kidneys were

smaller in size, pale to grey in color, was hard to cut and capsule peeled off with difficulty. The surface was uneven due to irregular contraction of the fibrous tissue “Small granular contracted kidney”. In glomerulonephritisin acute type, both the kidneys were enlarged and pale, capsule peeled off easily. In sub acute type, kidneys were enlarged, pale and smooth with non adherent capsule. In chronic type, kidneys were shrunken and contracted with finely granular surface.

Jeong *et al.*, (2006)at necropsyinvestigated canine renal failure syndrome in three dogs. They observed systemic hemorrhage in various organs including kidney. In addition to this hemorrhagic right kidney was smaller than the left kidney, and the left kidney had severe yellow-white mottled areas near the cranial part, and there was hemorrhage at the caudal part. They found green-yellowish colored nephroliths in the renal pelvis.

Heiene *et al.,* (2007) examined dogs with pyometra were examined for evidence of secondary renal damage and persisting renal disease through two retrospective studies. In Study 1, light microscopic lesions of renal tissue were graded and compared in nineteen dogs with pyometra and thirteen age-matched control bitches. In Study 2, forty-one owners of dogs with pyometra were interviewed approximately 8 years after surgery for evidence ofclinical signs of renal failure in order to document causes of death/euthanasia. They found out that interstitial inflammation and tubular atrophy were more pronounced in dogs with pyometra than in the control animals. Glomerular lesions classified as glomerular sclerosis were present in both groups. No unequivocal light microscopic features of glomerulonephritis were observed in bitches in any of the groups. Tubulointerstitial inflammation was observed, but glomerular damage beyond age-related changes could not be demonstrated by light microscopy in the dogs with pyometra. However, severe proteinuria after surgery may predispose to development of renal failure.

Chandrasekaran *et al.,* (2011) conducted 10 post mortem examinations of dogs that died during treatment for canine leptospirosis. Liver & kidneys were enlarged with varying degrees of vascular & degenerative changes.

Sangle (2014) found symmetrically small kidneys with thin granular cortex and increased peri pelvic fat in chronic glomerulonephritis.

2.7 Histopathological changes of renal disorders in dogs:

Wright *et al.,* (1976) stated that chronic renal disease is an important clinical problem in dogs. Until recently, diffuse renal fibrosis with chronic renal failure has been attributed mainly to chronic interstitial nephritis, itself considered to be the end stage of acute leptospiral nephritis. Morphologically, the two diseases (chronic interstitial and glomerular nephritis) appeared to be distinguishable on three grounds; the pattern and severity of fibrosis, the degree of fibrin deposition and the immunofluorescence findings.

Chew *et al.,* (1983)studied renal failure in 22 young Doberman Pinscher dogs. The kidneys were characterized pathologically by glomerular sclerosis, cystic glomerular atrophy, tubular dilatation, tubular atrophy, mononuclear interstitial inflammation, interstitial fibrosis, interstitial mineralization, and hyperplasia of the collecting duct epithelium.

Macdougall *et al.*, (1986) showed that in dogs, as in humans, glomerular disease is a common cause of chronic renal disease accounting for 52% of cases. Forty eight percent of cases were nonglomerular diseases. Interstitial nephritis, defined as chronic tubulo-interstitial disease without evidence of glomerulonephritis or pyelonephritis was less common than in earlier reports. Of the 40 cases of canine glomerular disease, 31 were classified as glomerulonephritis by currently accepted W.H.O. criteria used for human glomerulonephritis and six were amyloid. A classification similar to that of the W.H.O. has recently been proposed for animals by Winter and Majid. Mesangiocapillary glomerulonephritis has frequently been reported as a common type of canine glomerulonephritis. The histology showed typical hypercellularity, and capillary wall thickening due to mesangial interposition. An unusual feature in some of the dogs was the large size of the mesangial deposits in comparison with human glomerulonephritis. There were two cases of diffuse endocapillary proliferative glomerulonephritis, which has not previously been separated from other types of proliferative glomerulonephritis in the dog.

Eubig *et al.,* (2005) renal histopathology was evaluated in 16 dogs, and other tissues, including liver, heart, and lung, were evaluated in 10 of these dogs. The most consistent lesion present in biopsy or postmortem samples from 15 of 16 dogs was moderate to severe, diffuse renal tubular degeneration, especially in the proximal tubules. Proteinaceous and cellular debris often was present in dilated tubule lumens. They also found congestion and degenerative changes in liver as well as in other organs.

Ganti and Rao (2006) studied nephritis among animals. They observed following microscopical changes in different visceral organs in canine renal failures. I. Pyaemic nephritis – abscesses with leucocytic infiltration in the glomerular loop and in tubules. II. Pyelonephritis – purulent process with neutrophils and a few lymphocytes, which may be found as streaks among the tubules. III. Interstitial nephritis - In acute type, infiltration of lymphocytes and plasma cells, with less number of neutrophils in interstitial tissue. In chronic type, there was cystic dilatation of tubules with hyaline casts. Cirrhotic changes may be noticed in liver. IV. Glomerulonephritis - Acute type, there is increased cellularity of the glomeruli, sub acute type- proliferation of epithelial cells of the parietal layer of the bowman's capsule results in epithelial crescents, Chronic type – fibrosed glomeruli and disappearance of tubules.

Wakamatsu *et al.,* (2007) studied juvenile onset renal disease is described in 2 male and 2 female young Rottweiler dogs. Histologic changes in all dogs were cystic dilatation of Bowman's space, mesangialhypercellularity, and glomerulosclerosis. Three dogs also had glomerular crescents and moderate to severe interstitial fibrosis. Electron microscopy revealed glomerular basement membranes of variable thickness, with extensive splitting or lamellation of the lamina densa. These ultrastructural findings are similar to those found in people and in other breeds of dogs with inherited defects in type IV collagen.

Chandrasekaran *et al.,* (2011) conducted 10 post mortem examinations of dogs that died during treatment for canine leptospirosis. Histopathologically, liver revealed varying degrees of vascular & degenerative changes with scattered areas of mononuclear cell infiltration and kidney revealed vascular changes,

cystic dilatation, tubular necrosis with interstitial lymphoplasmacytic& neutrophilic infiltration.

George *et al.,* (2011) expressed that the presence of suspected primary glomerular disease is the most common and compelling reason to consider renal biopsy. Pathologic findings in samples from animals with nephritic or nephrotic glomerulopathies, as well as from animals with persistent subclinical glomerular proteinuria that is not associated with advanced chronic kidney disease, frequently guide treatment decisions and inform prognosis when suitable specimens are obtained and examined appropriately. Ultrasound-guided needle biopsy techniques generally are satisfactory; however, other methods of locating or approaching the kidney, such as manual palpation (e.g., in cats), laparoscopy, or open surgery, also can be used. Visual assessment of the tissue content of needle biopsy samples to verify that they are renal cortex (i.e., contain glomeruli) as they are obtained is a key step that minimizes the submission of uninformative samples for examination. Adequate planning for a renal biopsy also requires prior procurement of the fixatives and preservatives needed to process and submit samples that will be suitable for electron microscopic examination and immunostaining, as well as for light microscopic evaluation. Finally, to be optimally informative, renal biopsy specimens must be processed by laboratories that routinely perform the required specialized examinations and then be evaluated by experienced veterinary nephropathologists. The pathologic findings must be carefully integrated with one another and with information derived from the clinical investigation of the patient‟s illness to formulate the correct diagnosis and most informative guidance for therapeutic management of the animal‟s glomerular disease.

Sangle (2014) found large glomeruli with accentuation of lobules Irregular thickening of glomerular basement membrane by interposition of mesangial cells between endothelium and basement membrane Causes tram track / double contour appearance (PAS or silver stain), crescents in 20% Neutrophils often present May have hyaline aggregates of immune complexes in capillary lumina.

2.8 Histochemical findings in renal disorders in dogs:

Ramos-Vara *et al*., (2004) studied renal cysts in the cortex of a juvenile Belgian Malinois dog with acute renal failure were studied by means of light, scanning and transmission electron microscopy, immunohistochemistry for intermediate filaments, and binding for wheat germ agglutinin (WGA), peanut agglutinin (PNA), and Maclurapomifera agglutinin (MPA) lectins to determine the morphological and histochemical features of the epithelial cells of these cysts. The parietal podocytes expressed vimentin and cytokeratins and had affinity for WGA as do normal immature podocytes. These features suggest that the parietal podocytes are derived by metaplasia of the parietal cells. The basement membrane of Bowman's capsule was irregularly thickened and showed multifocal glycosylation changes with lectinhistochemistry (WGA, PNA, MPA) in areas adjacent to the parietal podocytes. Histologic and ultrastructural findings in this dog are consistent with glomerulocystic kidney disease.

Lavoue *et al.,* (2010) studied familial juvenile glomerulonephropathy (JGN) in several breeds of dogs. Clinical signs were typical of progressive glomerulopathy with resultant renal failure. They found increased blood urea nitrogen, creatinine and total cholesterol concentrations, and proteinuria in all patients. Affected dogs had abnormal kidney structure on abdominal ultrasound examination. Histopathologic examination revealed extensive cystic glomerular atrophy, glomerular hypercellularity, and capillary wall thickening without immune complex deposition when tested with immunohistochemistry or immunofluorescence. Electron microscopy did not disclose specific primary glomerular lesions. Mean age at death was 20 months and mean length of survival after diagnosis was 6 months. Both males and females from healthy parents were affected. An autosomal recessive mode of transmission is suspected, but a more complex mode of inheritance cannot be excluded.

3. MATERIALS AND METHODS

The present study was undertaken in the Department of Veterinary Pathology, Bombay Veterinary College, Mumbai for clinicopathological studies of canine renal dysfunction with special reference to estimation of lipocalin and beta 2 microglobulin biomarkers. Following materials were used and methods were employed for completion of this study.

3.1 Collection of clinical samples:

Fifty (50) clinical cases were collected from medical ward,BSPCA Hospital, Parel and private clinics in and around Mumbai from February to September, 2017. 50 Blood and urine samples were collected for further investigation and those cases were categorized clinically as follows, 1) Renal diseases (AKI; 30 and CKD; 14) and 2) Control (6). The dogs were considered healthy on the basis of physical examination, complete blood count, biochemistry profile and urinalysis. While collecting samples, the detailed information regarding age, sex and breed were noted by generating a proforma for clinical case record of each and every case.

3.2 Collection of blood samples:

Five milliliters of blood were collected from the cephalic or saphenous vein, using a dry sterile syringe and needle, and two milliliters was immediately transferred to a vial containing Ethylene Diamine-Tetra-Acetate (EDTA) as an anticoagulant. Three-millimeter blood was transferred to plane vial for serum sample, which was collected in three aliquots for evaluation of liver and renal function tests as well as estimation of lipocalin (NGAL) and beta 2 microglobulin biomarkers. Serum samples for estimation of biomarkers were stored at -80˚c until assayed (Schinstock, *et al*, 2012).

Table 1: Clinical case information

Case No.	Breed	Sex	Age (Years)	Clinical History of Dogs
01	GSD	Male	10	Anorexia, weakness, vomiting, anemia, oliguria, prognosis poor
02	ND	Male	08	Inappetence and vomiting of bile, anorexia, loose motion, dull, dark yellow urine, dehydration, prognosis poor
03	Pomeranian	Female	12	Anorexia, vomiting, dullness, unable to walk, increase in creatinine,
04	Doberman	Male	04	Anorexia, vomiting, increase in KFT and LFT, very dull and poor prognosis
05	Spitz	Female	13	Anorexia, vomiting, acute onset of azotemia
06	Labrador	Female	11	Anorexia, vomiting, cardiac enlargement, increase in KFT and LFT, dehydration, dyspnea, dullness, poor prognosis
07	Labrador	Male	1.2	Vomiting and loose motion 3-4 days, anorexia, increase KFT, prognosis poor
08	ND	Male	1.5	Anorexia, vomiting, AKI,
09	Labrador	Male	3.5	Sudden onset of azotemia, leptospirosis?
10	Labrador	Female	08	Vomiting, loose motion with blood, anorexia, increase creatinine
11	Labrador	Male	06	Efforts for urination, history of anuria/oliguria, acute onset of azotemia and icterus, dyspnea
12	Pomeranian	Female	15	Inappetence, vomiting, azotemia, weakness
13	Labrador	Male	10	Anorexia, vomiting, dyspnea, poor prognosis
14	Labrador	Female	05	Anorexia, vomiting, history of pyometra, weakness
15	Labrador	Male	3.5	Anorexia, oliguria, vomiting, loose motion, weakness, dehydration
16	Pomeranian	Female	09	Inappetence, vomiting, oliguria and proteinuria, weakness
17	Pomeranian cross	Female	06	Anorexia, vomiting, dehydration, loose motion, dullness
18	Labrador	Male	02	Vomiting, anorexia, increase KFT and LFT, anemic, Leptospirosis?
19	Labrador	Female	4.5	Anorexia, vomiting, hematuria, weakness
20	Labrador	Female	05	Inappetence, vomiting, loose motion, Increase in KFT

21	Pomeranian	Male	11	Inappetence, vomiting, loose motion
22	Pomeranian	Male	10	Inappetence, vomiting, loose motion, history of trypanosomiasis
23	Doberman	Male	7.5	Inappetence, vomiting, oliguria, sudden onset of azotemia
24	Labrador	Male	6.7	Inappetence, weakness, dehydration, anuria, sudden onset of AKI
25	ND	Male	09	Inappetence, vomiting, loose motion, Increase in KFT
26	Labrador	Male	02	Inappetence, vomiting, loose motion, Increase in KFT and LFT
27	Doberman	Female	13	Inappetence, increase in KFT and LFT, Oliguria, Thrombocytopenia
28	ND	Female	05	Inappetence, vomiting, unable to stand, weakness
29	Labrador	Male	6.5	Inappetence, vomiting, loose motion, Increase in KFT and LFT, dullness, anemia, oliguria
30	Labrador	Male	08	History of tick fever, anorexia, mild dehydration, anemic, hematemesis
31	ND	Male	03	Inappetence, very weak, dehydration, ulcers were present in mouth, azotemic smell
32	Labrador	Male	04	Inappetence, azotemia
33	Labrador	Male	10	Anorexia, vomiting, increase in KFT and LFT, Proteinuria
34	GSD	Male	09	Vomiting 3-4 times a day, anorexia, dehydration, dullness
35	GSD	Male	04	Anorexia, vomiting, polyuria followed by oliguria, severe dehydration, anemic, severe dullness, poor prognosis
36	Boxer	Female	07	Inappetence, vomiting, increase in KFT and LFT
37	Pomeranian	Female	07	Inappetence, increase in KFT, vomiting, dullness, anemia,
38	C. Spaniel	Female	08	Inappetence, increase in KFT, vomiting, dullness, anemia, polyuria
39	Pug	Male	06	Anorexia, severe dehydration and weakness, increase in KFT, vomiting
40	GSD	Male	10	Inappetence, azotemia
41	Labrador	Male	07	Anorexia, vomiting, hematuria, polyuria, anemic
42	Pug	Male	05	Anorexia, hematuria, vomiting
43	Pug	Male	10	Anorexia, hematemesis, hematuria, anemic, polyuria followed by anuria
44	ND	Female	08	Inappetence, polyuria, azotemia,

				weakness, dehydration
45	Doberman	Female	04	Healthy
46	Labrador	Male	6.5	Healthy
47	Rottweiler	Male	0.7	Healthy
48	Labrador	Male	05	Healthy
49	Labrador	Female	5.6	Healthy
50	Labrador	Female	02	Healthy

3.3 Hematological Parameters:

Hematological parameters were estimated with the help of automatic cell counter (Abacus- Diatron) by impedance method (Benjamin, 1985). The parameters studied were as follows,

1) Hemoglobin (Hb) (gm/dl),
2) Packed Cell Volume (PCV) (%),
3) Total Erythrocyte Count (TEC) (10^6 / cumm),
4) Total Leukocyte Count (TLC) (10^3 / cumm),
5) Platelet count (Lacs /cumm),
6) Mean corpuscular volume (MCV) (fl),
7) Mean corpuscular Hemoglobin (MCH) (pg),
8) Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl) and
9) Differential Leucocyte Count (DLC) (%).

3.4 Serum biochemistry:

The serum was separated by centrifuging the clotted blood in Eppendorf tubes, at 3000 rpm for 15 min. and following parameters were estimated by using semiautomatic biochemical analyzer (Erba- Chem.7), using commercial reagent

kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH) by following the procedure provided with respective kits.

3.4.1 Estimation of total serum protein (TSP) (gm/dl):

Total Protein was estimated by Biuret method (Tietz, 1986) by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

3.4.2 Estimation of serum albumin (ALB) (gm/dl):

Albumin was estimated by BCG method (Doumas *et al*., 1972) by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

3.4.3 Estimation of Serum Glutamic Pyruvic Transaminase (SGPT) (IU/L):

SGPT levels were estimated by IFCC method (Tietz, 1986) by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

3.4.4 Estimation of Serum Glutamic Oxaloacetic Transaminase (SGOT) (IU/L):

SGOT levels were estimated by IFCC method (Tietz 1986) by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

3.4.5Estimation of Total and Direct Bilirubin (BIT &BID) (mg/dl):

Total and Direct Bilirubin levels were estimated by Jendrassik and Grof method (Pearlman and Lee, 1974) by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

3.4.6 Estimation of Blood Urea Nitrogen (BUN) (mg/dl):

BUN levels were estimated by GLDH-UV Kinetics method **(**Tietz, 1976) by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

3.4.5 Estimation of creatinine (mg/dl):

Creatinine levels were estimated by modified Jaffe's method by using diagnostic kits from ROBONIK (INDIA) Pvt Ltd., Navi Mumbai (MH).

Staging of Kidney Diseases

Table 2: IRIS (International Renal Interest Society) AKI Grading Criteria

AKI Grade	Blood creatinine	Clinical Description
Grade I	<1.6 (mg/dl) (<140 µmol/l)	a. Documented AKI: (Historical, clinical, laboratory, or imaging evidence of acute kidney injury, clinical oliguria/anuria, volume responsiveness) and/or b. Progressive non azotemic increase in blood creatinine; ≥0.3 mg/dl (≥26.4 µmol/l) within 48 hours c. Measured oliguria (<1 ml/kg/hr) or anuria over 6 hrs
Grade II	1.7 – 2.5 mg/dl (141 – 220 µmol/l)	Mild AKI: a. Documented AKI and static or progressive azotemia b. Progressive azotemic increase in blood creatinine; ≥0.3 mg/dl ≥26.4 µmol/l) within 48 hours), or volume responsiveness‡ c. Measured oliguria (<1 ml/kg/hr) or anuria over 6 hrs
Grade III	2.6 – 5.0 mg/dl (221 – 439µmol/l)	Moderate to Severe AKI: a. Documented AKI and increasing severities of azotemia and functional renal failure
Grade IV	5.1 – 10.0 mg/dl (440 – 880 µmol/l)	
Grade V	>10.0 mg/dl (>880 µmol/l)	

Table 3: IRIS Staging of CKD:-

CKD Stage	Blood creatinine	Clinical Description
At Risk	<125µmol/l <1.4 mg/dl	History suggests the animal is at increased risk of developing CKD in the future because of a number of factors (such as, exposure to nephrotoxic drugs, breed, high prevalence of infectious disease in the area, or old age).
1	<125µmol/l <1.4 mg/dl	Nonazotemic. Some other renal abnormality present (such as, inadequate urinary concentrating ability without identifiable nonrenal cause, abnormal renal palpation or renal imaging findings, proteinuria of renal origin, abnormal renal biopsy results, increasing blood creatinine concentrations in samples collected serially)
2	125-180 µmol/l 1.4-2.0 mg/dl	Mild renal azotemia (lower end of the range lies within reference ranges for many laboratories, but the insensitivity of creatinine concentration as a screening test means that animals with creatinine values close to the upper reference limit often have excretory failure). Clinical signs usually mild or absent.
3	181-440µmol/l 2.1-5.0 mg/dl	Moderate renal azotemia. Many extrarenal clinical signs may be present.
4	>440µmol/l >5.0 mg/dl	Increasing risk of systemic clinical signs and uraemic crises

3.5 Estimation of Biomarkers:

3.5.1 Estimation of lipocalin (NGAL) by Enzyme Linked Immunosorbent Assay(ELISA)

The blood samples were allowed to clot for 10 to 20 minutes at room temperature and centrifuged at 2000-3000 rpm for 20 minutes.

The ELISA assay procedure was carried out as per the instructions given by the Canine NGAL ELISA kit **(Plate 1)**.

1. All reagents were brought to room temperature before use. The assay was performed at room temperature and dilute samples with sample diluent.

2. 100 µl standards/ calibrator dilution were added to the standard well (A to H) and 100 µl sample diluent added to one well as a blank.

3. 100 µl of diluted serum samples were added to sample wells in duplicates, the plate was covered with a sealer and incubated at room temperature for 60 minutes.

4.The sealer was removed and plate was washed 3 times with 300 µl wash buffer each time. After that the plate was blotted onto a paper towel.

5. 100 µl Biotinylated Dog-NGAL Antibody was added to each well. Plate was covered with a new sealer and incubated for 60 minutes at room temperature.

6. The sealer was removed and plate was washed 3 times with 300 µl wash buffer each time. After that the plate was blotted onto a paper towel.

7. 100 µl HRP-Streptavidin was added to each well. Plate was covered with a new sealer and incubated for 60 minutes at room temperature.

8. The sealer was removed and plate was washed 3 times with 300 µl wash buffer each time. After that the plate was blotted onto a paper towel.

9. 100 µl TMB Substrate was added in each well. Plate was covered with a new sealer and incubated for 10 minutes at room temperature in the dark which developed blue color by reaction between HRP streptavidin and TMB substrate.

10. 100 µl stop solution was added to each well where the blue color changed to yellow immediately.

11.The Optical Density (OD) value of each well was measured immediately using a microplate reader set at 450 nm.

3.5.2 Estimation of beta 2 microglobulin by Enzyme Linked Fluroscent Assay (ELFA)

The assay principles combine a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

1. Allow all the reagents to come at room temperature for at least 30 minutes.
2. Use one B2M strip and one B2M SPR for each sample, control or calibrator to be tested.
3. Type or select B2M to enter the test code. The calibrator must be identified by S1 and tested in duplicate.
4. Mix the calibrator, controls and samples using a vortex type mixer.
5. Pipette 100 µl of calibrator, sample or control into the sample well.
6. Insert the SRPs and strips into the instrument.
7. Initiate the assay, all the assay steps are performed automatically by instrument. The assay will be completed within approximately 40 minutes.

3.6 Urine analysis

Urine samples were collected in a sterile container and examined for routine urine analysis. (Benjamin, 1985). Following parameters were studied

1) Physical Examination of urine
2) Chemical Examination of urine
3) Microscopic Examination of urine

3.6.1 Physical Examination of urine :

Colour, odour, pH and transparency of urine samples were evaluated. (Benjamin, 1985).

3.6.2 Chemical Examination of urine :

Protein in urine is estimated by routine chemical examination of urine. The test is known as Heller's Nitric Acid ring test. Procedure is as follows,

1. Take a sterile test tube and add 3 ml of conc.HNO_3.

2. Add 3 ml of urine in conc. HNO_3 in such a way that urine would not get mixed with HNO_3 and forms a separate, distinct layer over it.

Interpretation:- White colour ring at the junction of two layers confirms the presence of protein in urine (Benjamin, 1985).

3.6.3 Microscopic examination of urine sediment:

The well mixed urine samples in Eppendorf tube were centrifuged for 10 minutes at 1000 – 2000 rpm. The supernatant was discarded and Eppendorf tube was shaken to break up the sediment and one drop of it is taken on the glass slide and covered with coverslip. The unstained slide is examined immediately under low power (10 X objective) and high power (40 X objective) of light microscope according to the method given by Brar (2000). Smear will be prepared of the sediment and stained with the field stain for further examination.

3.7 Post mortem and gross examination

Two post mortem cases of dogs suspected for renal disorders were selected for this study. Detailed postmortem examination was carried out. Piece of kidney and liver of clinical cases were collected in 10 percent neutral buffered formalin for histopathological examination.

3.8 Histological staining and classification

Tissue samples of kidneys and liver of clinical cases were dehydrated in ascending grade of alcohol cleared in xylene and embedded in paraffin wax. Four to five micron thick sections were stained with routine hematoxylin and eosin-staining method by Luna, (1968) and tissue were examined.

3.9Histochemistry:

For histochemistry Masson's trichrome staining and van kossa staining were performed.

3.10Statistical analysis

All the data recorded during the study was subjected to statistical analysis by applying Completely Randomized Design (CRD) as suggested by Snedecor and Cochran, (2004).

4. RESULT AND DISCUSSION

The present study entitled withclinicopathological studies of canine renal dysfunction with special reference to lipocalin (NGAL) and beta 2 microglobulin (B2M) biomarkers was undertaken in the Department of Veterinary Pathology, Bombay Veterinary College, Parel, Mumbai to find out the incidence rate of canine renal dysfunction in and around Mumbai, to asses clinicopathology of renal failure by using biomarkers such as lipocalin and beta 2 microglobulin.

Fifty (50) cases of renal failure in dogs from different breed, sexwas included in this study. Dogs were categorized in 3 groups,**acute kidney failure** on the basis of symptoms of acute kidney failure include lethargy, poor or absent appetite, decrease in the frequency and amount of urination, vomiting and hemato-biochemical observations. **Chronic kidney failure** on the basis of Depression, Weight loss, Increased thirst, Lack of appetite (anorexia), Blood in the urine (hematuria), increase in the frequency and amount of urination and hemato-biochemical observations. Healthy dogs were selected on the basis of clinical examination and hemato-biochemical observations.Serum samples were collected from dogs having acute kidney failure (30) and chronic kidney failure (14). 6 serum samples were collected from healthy dogs.

Result and discussion is as follows according to chronology of research work,

4.1 Incidence of canine real disorders with respect to age, breed and sex

4.2 Haematological alterations in canine renal disorders

4.2.1 Hb

4.2.2 PCV

4.2.3 TEC

4.2.4 Erythrocytic indices (MCV, MCH & MCHC)

4.2.5 TLC

4.2.6 DLC

4.3 Biochemical alterations in canine renal disorders

4.3.1 LFT

4.3.2 KFT

4.4 Urine analysis

4.5 Estimations of renal biomarkers in canine renal disorders

4.5.1 Lipocalin (NGAL) estimations by ELISA method

4.5.2 Beta 2 microglobulin estimations by ELFA method

4.6 Gross lesions of renal disorders in dogs

4.7 Histopathological examinations

4.1 Incidences of canine renal disorders with respect to age, sex and breed

Serum biochemistry data of the clinical cases of renal disorders (serum creatinine> 1.5 mg/dl) over a period of two years (2015- 2017) were studied in order to record the incidence of renal failure cases. Over a period of 8 months (February 2017- September 2017), total of fifty clinical samples and three post mortem cases were considered for this study. On the basis of clinical signs, clinical cases were divided into three groups, on the basis of age, clinical cases were divided in three groups and on the basis of breeds, clinical cases were divided into five groups.

4.1.1 Age wise incidence:

On the basis of departmental survey of clinical samples of last five years, observations of the total and percent population affected in relation to different age groups are presented in **Table 4**. The age of the dogs affected with canine renal disorders in the survey study varied from 1 year to 11 years. Highest incidence was recorded in the age group of 6-10 years (66.67%), followed by 0-5 years of age (22.22%) and11-15 years (11.11%).

Observations of the clinical samples in relation to different age groups are presented in **Table 5**. The age of the dogs affected with canine renal disorders in the present study varied from 0.5 years to 11 years. Highest incidence was recorded in the age group of 6-10 years (52.27%; ARF(73.33%) and CRF(26.67%)), followed by 0-5 years of age (34.1%; ARF(56.52%) and CRF(43.48%)) and lowest in the age group of 11-15 years (ARF:13.63%) respectively.

These findings are in accordance with findings of Srinivasan *et al.* (1993) found that out of 50 canine patients diagnosed with primary renal insufficiency, 52% were in the age group of 5-10 years, 36% were more than 10 years and 12% were less than 5 years old. Polzin *et al.,* (2005), they had reported the mean age of diagnosis of chronic renal failure in dogs of 6.5 years. Mrudula *et al*., (2005) found that the mean age of affected animals was 7.5 years.

This might be due to the fact that with advancing age blood flow to the kidney decreases and there is a loss of nephrons, the resorption process with in the prevailing nephrons also gets impaired with advance in age (Grauer and Lane,1995).

Yhee (2010) reported that the 5-6 years of age was most common age to be affected with chronic renal failure. Bartlett *et al*., (2010) found that the chronic kidney disease in dogs occurs at the average 9.9 years of age. Sharma and Shrestha (2012) reported that 5-10 years age group found to be more vulnerable for renal disorders, followed by 10-15 years and 0-5 years old. Kandula and Karlapudi (2014) found prevalence of renal failure was higher in dogs of above 8 years of age as compared to dogs upto 4 years of age. This is in agreement with the present study.

4.1.2 Sex wise incidence:

On the basis of departmental survey of clinical samples of last two years, observations of the total and percent population affected in relation to different age groups are presented in **Table 4**. In the survey study, out of 27 cases, 18 cases were recorded in males (66.67%) and 9 cases were recorded in females (33.33%).

Observationsof the clinical samples in relation to sex are presented in **Table 5.** In the present study, out of 44 cases, 28 cases were recorded in males (63.63%; ARF(64.29%) and CRF(35.71%)) and 16 cases were recorded in females (36.37%; ARF(75%) and CRF(25%)).

These findings of the present study were supported by Srinivasan *et al.* (1993), Vaden *et al*., (1997), Sastry and Rama Rao (2001), Mrudula *et al*., (2005), Vegad (2005), Sharma and Shrestha (2012) and Oburai *et al,* (2015) they found that prevalence of renal insufficiency was more in male dogs than female dogs. Higher prevalence of renal failure inmale dogs could be due to more risk associated withurolithiasis in male than female due toanatomic characteristics as male dog has long urethra along with OS-penis (Bjorling, 2003).

Whereas, Kandula and Karlapudi (2014[a]) found prevalence of renal insufficiency in female dogs(63.16%). This might be due to various unhygienic managemental practices during puerperal stage that may lead to genital infection, which may progress as urogenital complaints (Tilley and Smith, 2007).

4.1.3 Breed wise incidence:

On the basis of departmental survey of clinical samples of last five years, observations of the total and percent population affected in relation to different age groups are presented in **Table 4**. In this survey study, maximum incidence

was observed in Labradors (29.62%) and non-descript breeds (25.92%) followed by Pomeranian (11.11%) and German shepherd (3.70%).

Observations of the clinical samples in relation to different breeds are presented in **Table 5**. In this study, maximum incidence was observed in Labradors (40.90%) and Pomeranian breeds (15.90%) followed by non-descript (13.63%), and German shepherd (9.09%).

Higher incidence of renal disorders is seen in Labrador. It might be due to inadequate knowledge about the feeding (giving more than required amount of protein) (Sharma and Shrestha, 2012) and distribution of a particular breed in the geographical area where the study was carried out.

Kandula and karlapudi (2014[a]) reported high prevalence of renal insufficiency inbreeds affected were Labrador retriever (21.05%) followed by German shepherd (15.79%) and Boxer, Doberman pinscher, Pomeranian and Pug (5.26%, each). Tufani *et al.,* (2015) recorded highest incidence in Labrador (18%) followed by Pomeranian (16%).

Table 4: Incidence of renal disorders in canines- Departmental survey (2015 - 2017)

Age	Total cases	Creatinine (1.5- 5 mg/dl)	Creatinine (above 5 mg/dl)
0-5	6	4	2
6-10	18	14	4
10-15	3	1	2
Total	**27**	**19**	**8**
Sex			
Male	18	14	4
Female	9	5	4
Total	**27**	**19**	**8**
Breed			
Pomeranian	3	2	1
Non-descript	7	3	4
German shepherd	1	1	0
Labrador	8	7	1
Other Species	8	6	2
Total	**27**	**19**	**8**

Table 5: Summary of Incidence of renal disorders in canines(clinical cases)

Age	Total cases	ARF	CRF	Healthy
0-5	19	11	04	4
6-10	25	13	10	2
10-15	06	06	00	00
Total	**50**	**30**	**14**	**6**
Sex				
Male	31	18	10	3
Female	19	12	4	3
Total	**50**	**30**	**14**	**6**
Breed				
Pomeranian	7	6	1	0
Non-descript	6	4	2	0
German shepherd	4	1	3	0
Labrador	22	15	3	4
Other Species	11	4	5	2
Total	**50**	**30**	**14**	**6**

4.2 Hematological alteration in canine renal disorder

The mean value of hemoglobin (Hb) were 10.60 ± 0.55, 7.75 ± 0.16 and12.20 ± 0.42, Total erythrocyte count (TEC) as 5.01 ± 0.24, 3.72 ± 0.13 and 5.6 ± 0.14 and Packed cell volume (PCV) as 33.43 ± 1.47, 23.34 ± 4.0.68 and 36.02 ± 0.99 respectively for groups ARF, CRF and Healthy. The mean of MCV were64.19 ± 1.25, 66.88 ± 1.16 and 65.74 ± 1.05, MCH were 22.08 ± 0.38, 21.74 ± 0.48 and 21.56 ± 0.18 and MCHC were 32.35 ± 0.47, 32.82 ± 0.41 and 33.12 ± 0.53respectively for groups ARF, CRF and Healthy. The mean of mean of TLC were 18.5 ±1.23, 19.75 ± 2.7 and 12.99 ± 1.68 respectively for ARF CRF and Healthy groups.

The observations of mean ± S.E. values of Hb, PCV, TEC, MCV, MCH, MCHC, TLC, Neutrophils and platelets of renal failure dogs were given in **Table 6,7,8,9** and **figure 1 to 6.**

There was statistically significant (P ≤ 0.05) difference in the values of Hb, PCV, TEC and TLC values in different groups **(Table 10).**

In the present study, out of 50 clinical cases, 24 cases (48%) showed reduction in the values of Hb, 22 cases (44%) showed reduction in values of PCV and 22 cases (44%) showed reduction in TEC values.

There was no significant reduction in the values of MCH but 16 and 9 cases showed reduction in the values of MCV (32%) and MCHC (18%) respectively in the cases of renal failure in dogs.

Out of 50 clinical cases 14 cases (28%) showed reduction in the value of platelets. 24 cases(48%) showed increase in the value of TLC. There were28 cases (56%) which showed increase in the value of neutrophils.

There were statistically non-significant(P ≤ 0.05) variations in hematological parameters within different breeds of dogs **(Table 11)** anddifferent age group **(Table 12).**

Decrease in Hb (anemia), PCV and TEC in dogs suffering from renal failure might be due to degeneration of tubular epithelium along with fibrous tissue proliferation in interstitial space as well as around glomeruli. Due to this there is less production of erythropoietin from juxtaglomerular apparatus which ultimately leads to reduction in the erythropoiesis (Cowgill *et al.* 1998) and increase in TLC and neutrophils might be due to various inflammatory lesions in kidneys and other systems of the body (Senior *et al*, 1986).

These findings were in accordance with the reports of previous studies by Wright *et al*., (1976), Miyamoto *et al.,* (1997), Robertson and Seguin (2008), Polzin (2011) and Sperschneider (1977) recorded anemia in chronic renal insufficiency. Picut (1985), Cowgill (1992) and Oishi *et al*., (1995) observed decrease in hemoglobin, TEC and PCV values in cases of renal insufficiency. Whereas, Bradea, *et al*., (2013), Kandula and Karlapudi (2015) and Oburai *et al.* (2015) found that there was decrease in values of Hb, PCV and TEC and increase in leukocytes along with neutrophilia.

Table 6: Mean values of Hematological Parameters in clinical cases: (Group wise)

Parameters		Group of Animals		
		ARF	**CRF**	**Healthy**
Hb (g/dl)		10.60 ± 0.55[a]	7.75 ± 0.16[b]	12.20 ± 0.42[a]
PCV (%)		33.43 ± 1.47 [a]	24.34 ± 0.69[b]	36.027 ± 0.99 [a]
TEC (10^6/cumm)		5.01 ± 0.24[a]	3.72 ± 0.13[b]	5.603 ± 0.14[a]
MCV (fl)		64.19 ± 1.25^{NS}	66.88 ± 1.16^{NS}	65.748 ± 1.05^{NS}
MCH (pg)		22.08 ± 0.37^{NS}	21.74 ± 0.48^{NS}	21.560 ± 0.18^{NS}
MCHC (g/dl)		32.35 ± 0.47^{NS}	32.82 ± 0.41^{NS}	33.125 ± 0.53^{NS}
TLC (10^3/cumm)		18.50 ± 1.23[a]	19.75 ± 2.7[a]	12.997 ± 1.68[b]
DLC	N (%)	79.20 ± 1.45^{NS}	76.50 ± 2.61^{NS}	72 ± 2.460^{NS}
	L (%)	18.200± 1.28^{NS}	20.214± 2.7^{NS}	24.33± 2.37^{NS}
PLT(Lacs/cumm)		2.49 ± 0.27^{NS}	2.078 ± 0.52^{NS}	3.022 ± 0.52^{NS}

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 7: Mean values of Hematological Parameters in clinical cases: (Age wise)

Parameters		Group of Animals		
		0-5 years	**6-10 years**	**11-15 years**
Hb (g/dl)		11.16 ± 0.81^{NS}	10.069 ± 0.63^{NS}	11.25 ± 1.08^{NS}
PCV (%)		33.87 ± 2.36^{NS}	32.44 ± 1.8^{NS}	34.67 ± 3.07^{NS}
TEC (10^6/cumm)		5.27 ± 0.36^{NS}	4.73 ± 0.29^{NS}	5.28 ± 0.45^{NS}
MCV (fl)		64.87 ± 1.8^{NS}	64.6 ± 1.25^{NS}	67.22 ± 2.13^{NS}
MCH (pg)		22.17 ± 0.49^{NS}	22.61 ± 0.39^{NS}	22.9 ± 0.92^{NS}
MCHC (g/dl)		33.38 ± 0.42^{NS}	31.93 ± 0.54^{NS}	32.47 ± 0.85^{NS}
TLC (10^3/cumm)		21.76 ± 2.12^{NS}	17.36 ± 1.74^{NS}	17.61 ± 0.59^{NS}
DLC	N (%)	80.67± 2.25^{NS}	76.78 ± 1.85^{NS}	78.5 ± 2.06^{NS}
	L (%)	16.8± 2.07^{NS}	20.35 ± 1.85^{NS}	18.17± 1.18^{NS}
PLT(Lacs/cumm)		2.85 ± 0.5^{NS}	2.47 ± 0.24^{NS}	3.32 ± 0.93^{NS}

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 8: Mean values of Hematological Parameters in clinical cases: (Breed wise)

Parameters		Breed		
		Labrador	German Shephard	Nondescript
Hb (g/dl)		9.91 ± 0.69^{NS}	8.07 ± 1.38^{NS}	13.08 ± 1.24^{NS}
PCV (%)		31.72 ± 1.79^{NS}	25.47 ± 4.83^{NS}	40.11 ± 3.58^{NS}
TEC (10^6/cumm)		4.75 ± 0.32^{NS}	3.91 ± 0.78^{NS}	6.03 ± 0.48^{NS}
MCV (fl)		64.58 ± 1.61^{NS}	67.26 ± 2.92^{NS}	63.17 ± 3.27^{NS}
MCH (pg)		21.64 ± 0.34^{NS}	22.03 ± 1.44^{NS}	21.46 ± 0.63^{NS}
MCHC (g/dl)		32.04 ± 0.68^{NS}	33.62 ± 1.03^{NS}	33.62 ± 0.65^{NS}
TLC (10^3 / cumm)		18.77 ± 1.73^{NS}	24.76 ± 1.84^{NS}	20.46 ± 3.75^{NS}
DLC	N (%)	76.83 ± 1.93^{NS}	72.75 ± 7.78^{NS}	81.83 ± 1.92^{NS}
	L (%)	20.78 ± 1.77^{NS}	24± 7.3^{NS}	16± 2.4^{NS}
PLT (Lacs/ cumm)		2.17 ± 0.21^{NS}	3.45 ± 1.55^{NS}	3.16 ± 0.62^{NS}

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 9: Mean values of Hematological Parameters in clinical cases: (Breed wise)

Parameters		Breed	
		Pomeranian	Other breeds
Hb (g/dl)		10.51 ± 0.76^{NS}	11.51 ± 0.9^{NS}
PCV (%)		31.31 ± 1.95^{NS}	36.58 ± 2.66^{NS}
TEC (10^6/cumm)		4.80 ± 0.37^{NS}	5.40 ± 0.36^{NS}
MCV (fl)		64.35 ± 1.66^{NS}	66.79 ± 1.44^{NS}
MCH (pg)		22.89 ± 1.04^{NS}	22.25 ± 0.56^{NS}
MCHC (g/dl)		32.57 ± 0.75^{NS}	32.06 ± 035^{NS}
TLC (10^3 / cumm)		14.67 ± 1.33^{NS}	18.81 ± 3.36^{NS}
DLC	N (%)	77.85 ± 1.74^{NS}	81.88 ± 2.3^{NS}
	L (%)	18.71± 1.2^{NS}	14.66± 2.29^{NS}
PLT (Lacs/ cumm)		2.56 ± 0.37^{NS}	3.32 ± 0.66^{NS}

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 10: Analysis of variance of Hematological parameters in clinical cases: (Group wise)

Parameters	Source of variation	Df	SS	MS	F cal	CD value at 5%
Hb (g/dl)	Treatments	2	124.349	62.175	11.910	2.654*
	Error	47	245.350	5.220	-	
	Total	49	-	-	-	
PCV (%)	Treatments	2	947.246	473.623	10.707	7.726*
	Error	47	2078.994	44.234	-	
	Total	49	-	-	-	
TEC (10^6 /cumm)	Treatments	2	22.360	11.180	10.174	1.218*
	Error	47	51.649	1.099	-	
	Total	49	-	-	-	
MCV (fl)	Treatments	2	71.481	35.741	0.975	NS
	Error	47	1723.700	36.674	-	
	Total	49	-	-	-	
MCH (pg)	Treatments	2	2.000	1.000	0.266	NS
	Error	47	176.438	3.754	-	
	Total	49	-	-	-	
MCHC (g/dl)	Treatments	2	4.229	2.114	0.397	NS
	Error	47	250.199	5.323	-	
	Total	49	-	-	-	
TLC (10^3 /cumm)	Treatments	2	455.291	227.646	3.796	8.995*
	Error	47	2818.463	59.967	-	
	Total	49	-	-	-	
N (%)	Treatments	2	281.880	140.940	1.952	NS
	Error	47	3394.300	72.219	-	
	Total	49	-	-	-	
PLT (10^3 /cumm)	Treatments	2	3.956	1.978	0.614	NS
	Error	47	151.378	3.221	-	
	Total	49	-	-	-	

NS Non-significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

Table 11: Analysis of variance of Hematological parameters in clinical cases:(Age wise)

Parameters	Source of variation	Df	SS	MS	F cal	CD value at 5%
Hb (g/dl)	Treatments	2	13.754	6.877	0.699	NS
	Error	41	403.515	9.842	-	
	Total	43	-	-	-	
PCV (%)	Treatments	2	32.987	16.494	0.204	NS
	Error	41	3314.336	80.837	-	
	Total	43	-	-	-	
TEC (10^6/cumm)	Treatments	2	3.296	1.648	0.827	NS
	Error	41	81.714	1.993	-	
	Total	43	-	-	-	
MCV (fl)	Treatments	2	33.502	16.751	0.399	NS
	Error	41	1719.200	41.932	-	
	Total	43	-	-	-	
MCH (pg)	Treatments	2	8.783	4.391	1.075	NS
	Error	41	167.485	4.085	-	
	Total	43	-	-	-	
MCHC (g/dl)	Treatments	2	18.991	9.495	1.745	NS
	Error	41	223.065	5.441	-	
	Total	43	-	-	-	
TLC (10^3 /cumm)	Treatments	2	185.943	92.971	1.449	NS
	Error	41	2630.353	64.155	-	
	Total	43	-	-	-	
N (%)	Treatments	2	137.140	68.570	0.904	NS
	Error	41	3108.746	75.823	-	
	Total	43	-	-	-	
L (%)	Treatments	2	117.436	58.718	0.850	NS
	Error	41	2832.451	69.084	-	
	Total	43	-	-	-	
PLT (Lacs/cumm)	Treatments	2	3.778	1.889	0.654	NS
	Error	41	118.433	2.889	-	
	Total	43	-		-	

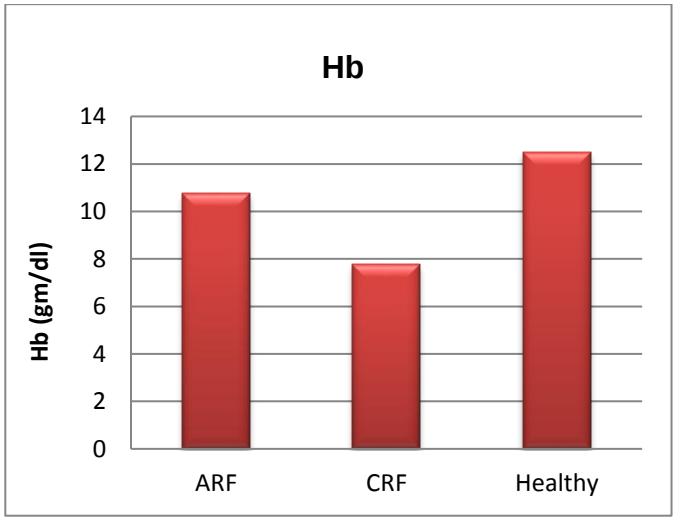
NS Non-significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

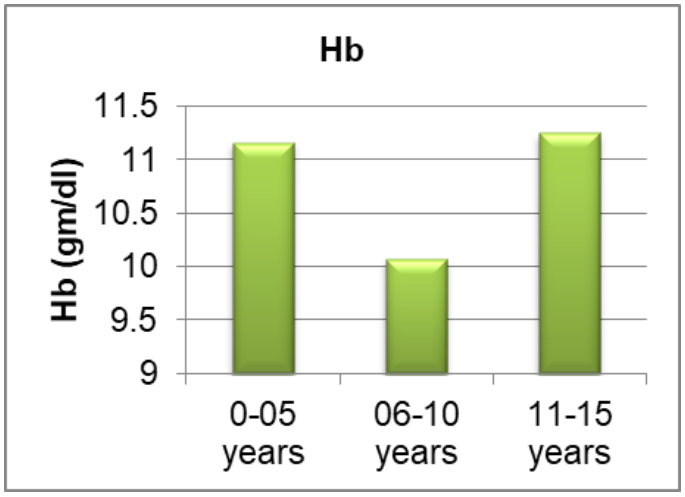
Table 12: Analysis of variance of Hematological parameters in clinical cases: (Breed wise)

Parameters	Source of variation	Df	SS	MS	F cal	CD value at 5%
Hb (g/dl)	Treatments	4	78.317	19.579	2.253	NS
	Error	39	338.952	8.691	-	
	Total	43	-	-	-	
PCV (%)	Treatments	4	692.536	173.134	2.543	NS
	Error	39	2654.787	68.071	-	
	Total	43	-	-	-	
TEC (10^6 /cumm)	Treatments	4	13.937	3.484	1.912	NS
	Error	39	71.073	1.822	-	
	Total	43	-	-	-	
MCV (fl)	Treatments	4	75.338	18.835	0.438	NS
	Error	39	1677.364	43.009	-	
	Total	43	-	-	-	
MCH (pg)	Treatments	4	10.132	2.533	0.595	NS
	Error	39	166.135	4.260	-	
	Total	43	-	-	-	
MCHC (g/dl)	Treatments	4	19.042	4.760	0.832	NS
	Error	39	223.014	5.718	-	
	Total	43	-	-	-	
TLC (10^3 /cumm)	Treatments	4	277.644	69.411	1.06	NS
	Error	39	2538.652	65.094	-	
	Total	43	-	-	-	
N (%)	Treatments	4	354.057	88.514	1.194	NS
	Error	39	2891.829	74.149	-	
	Total	43	-	-	-	
L(%)	Treatments	4	379.347	94.837	1.439	NS
	Error	39	2570.540	65.911	-	
	Total	43	-	-	-	
PLT (Lacs/cumm)	Treatments	4	11.896	2.974	1.05	NS
	Error	39	110.315	2.829	-	
	Total	43	-	-	-	

^{NS}Non-significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

Figure 1: Mean values of Hb in clinical cases: (Group wise, Age wise, Breed wise)





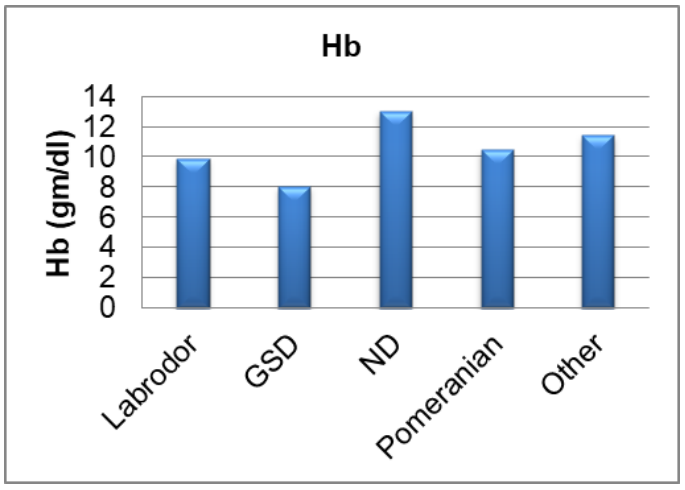
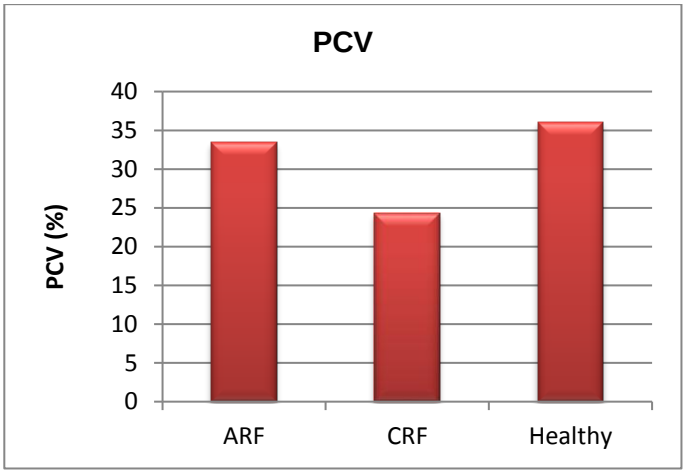
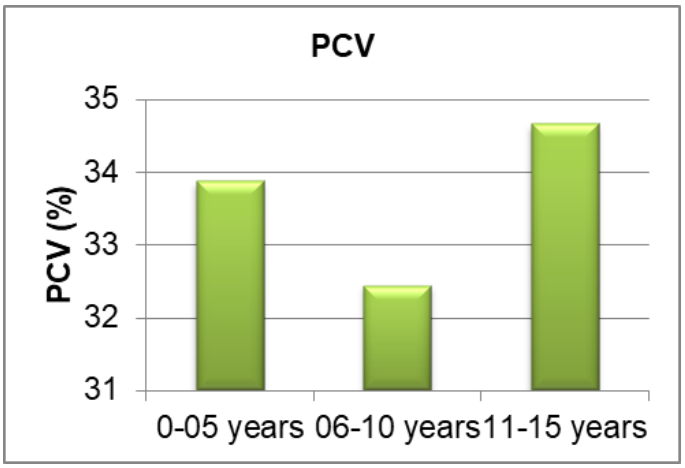


Figure 2: Mean values of PCV in clinical cases: (Group wise, Age wise, Breed wise)





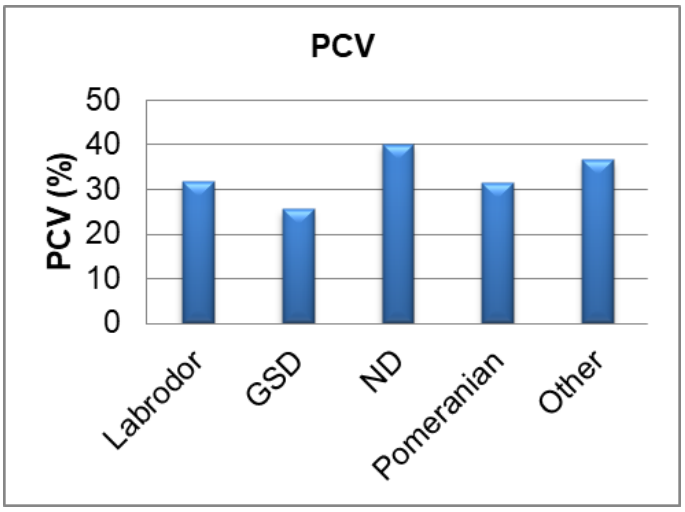
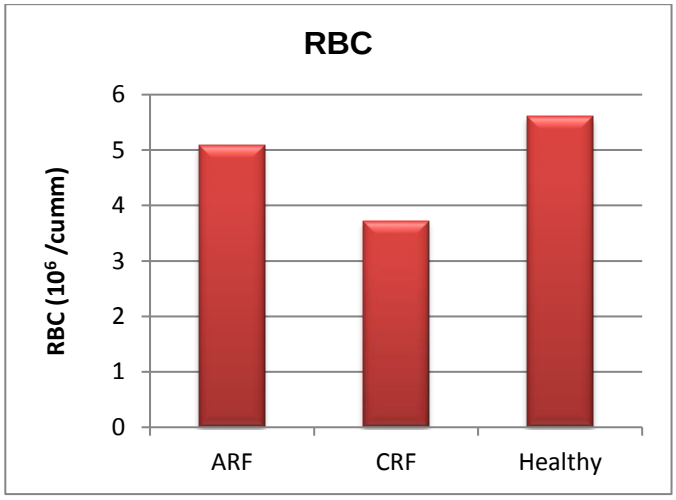
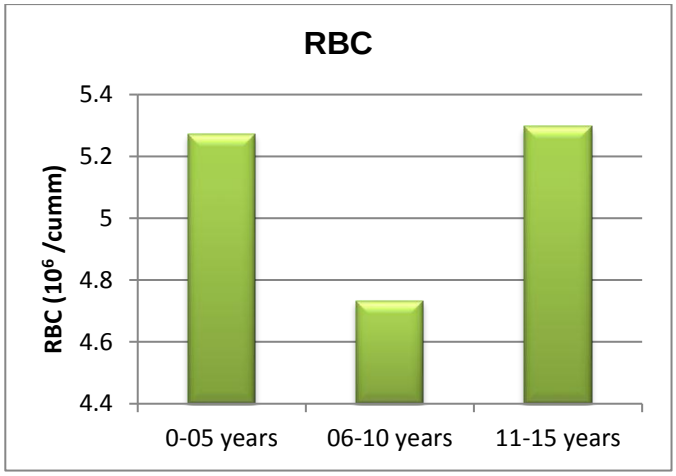


Figure 3: Mean values of RBC in clinical cases: (Group wise, Age wise, Breed wise)





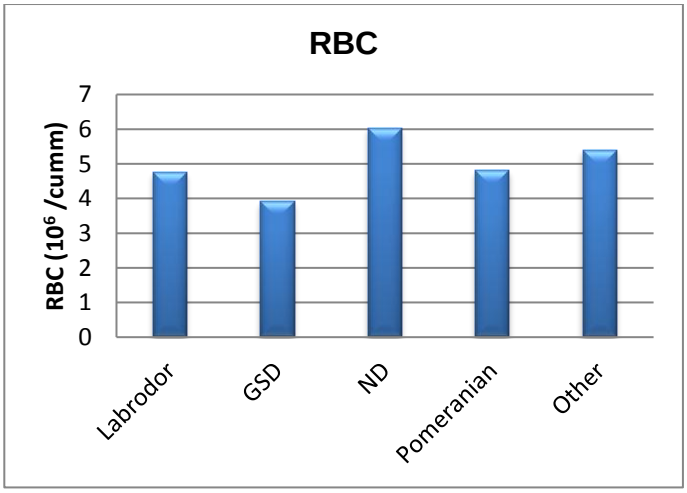
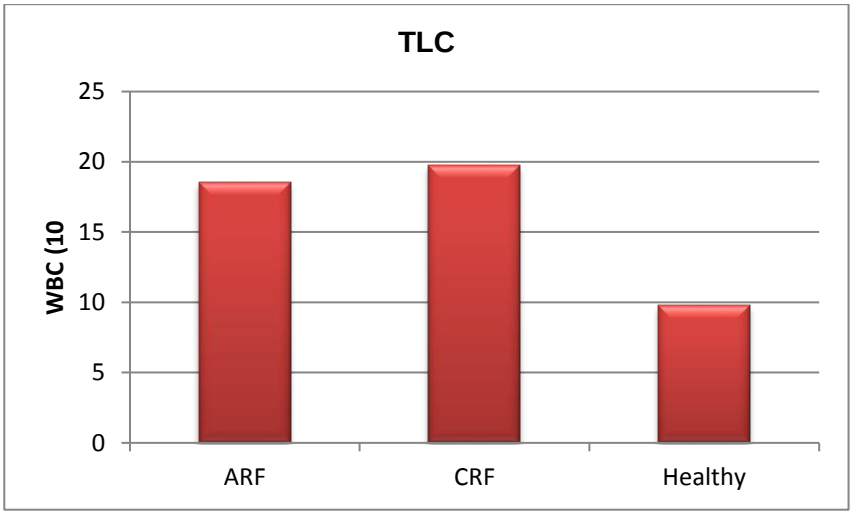
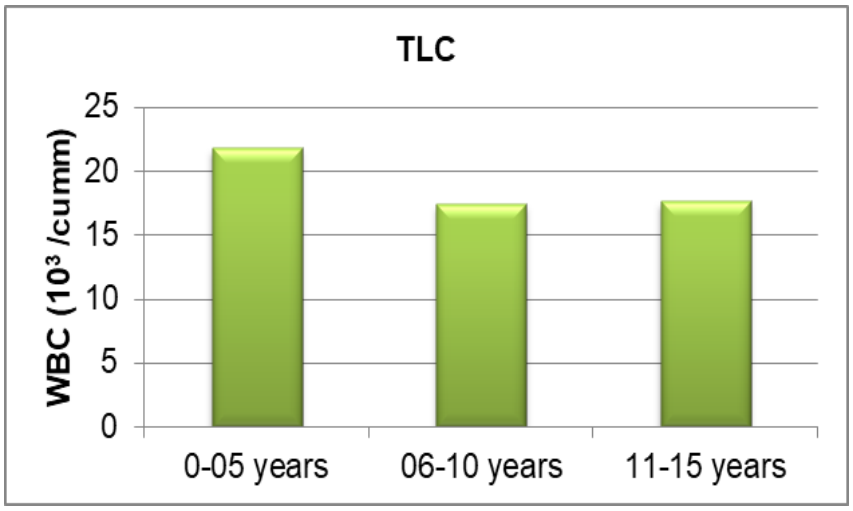


Figure 4: Mean values of TLC in clinical cases: (Group wise, Age wise, Breed wise)





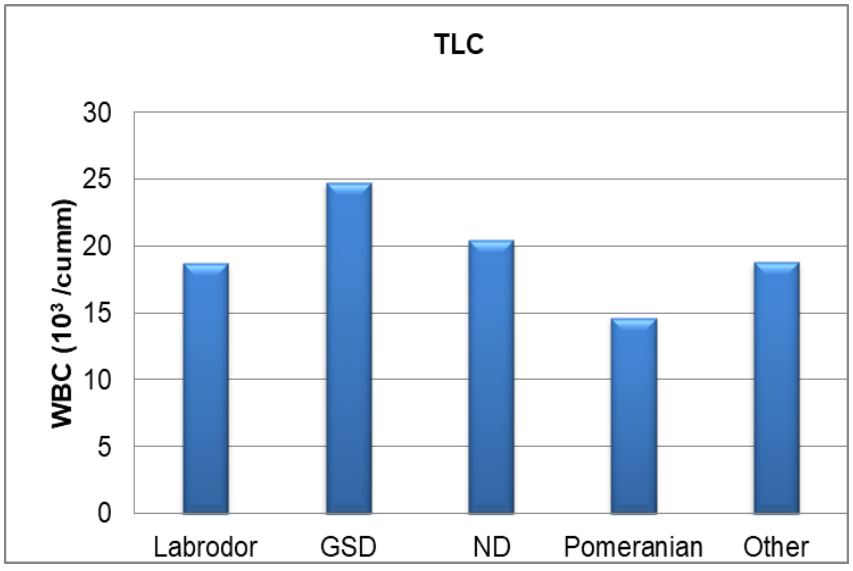


Figure 5: Mean values of Neutrophils in clinical cases: (Group wise, Age wise, Breed wise)

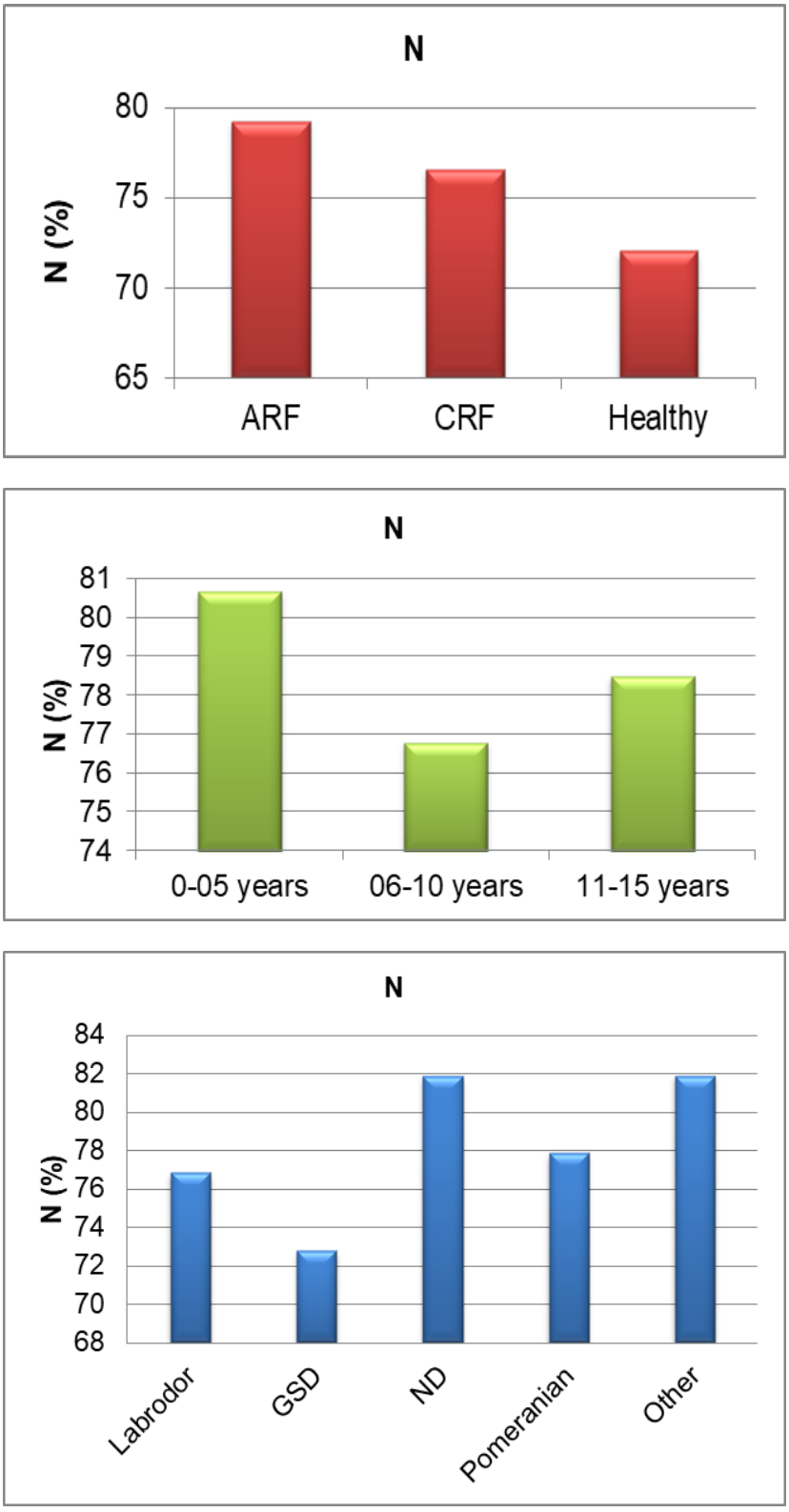
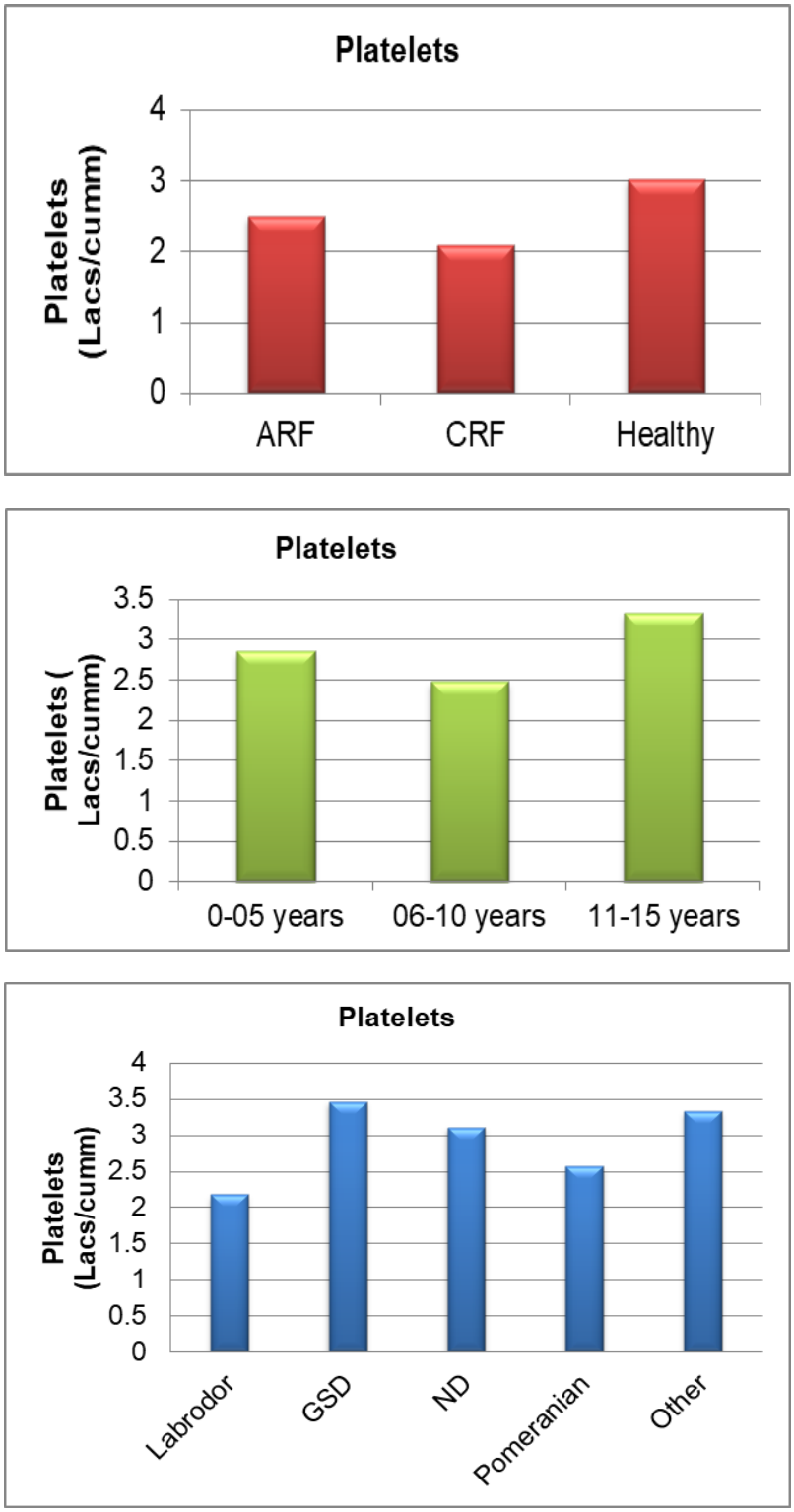


Figure 6: Mean values of platelets in clinical cases: (Group wise, Age wise, Breed wise)



4.3 Biochemical alterations in canine renal disorders

In the present study, the mean of BUN and creatinine was 114.2 ± 15.66, 91.75 ± 22, 20.46 ± 2.45 and 10.74 ± 0.96, 4.42 ± 0.89, 1.05 ± 0.13 respectively for ARF, CRF and Healthy groups. The mean of SGOT was 73.37 ± 9.89, 42. 96 ± 3.58 and 41.27 ± 3.41, SGPT was 88.44 ± 9.89, 62.67 ± 7.9 and 50.04 ± 4.76, ALP was 358.55 ± 121.07, 132.38 ± 15.3 and 108.8 ± 29.27, TB was 1.43 ± 0.35, 056 ± 0.2 and 0.62 ± 0.13, TP was 6.36 ± 0.18, 6.59 ± 0.16 and 6.42 ± 0.34, ALB was 2.58 ± 0.08, 2.55 ± 0.15 and 2.61 ± 0.22 respectively for ARF, CRF and Healthy groups.

The mean ± SE values of SGPT, SGOT, ALP, serum total protein, serum albumin, serum globulin, TB, DB, IB, BUN and serum creatinine in clinical cases of canine renal failure according to group wise, age wise and breed wise were recorded in **Table 13, 14, 15, 16** respectively and graphical representation of these values was given in **Figure 7 to 14**.

There was statistically significant (P ≤ 0.05) variation in the values of BUN and creatinine within the groups **(Table 17)**. These findings might be due because of ARF and CRF there might be damage to kidney leading to increase in BUN and serum creatinine level.

In the present study, out of 50 clinical cases, 10 cases (20%) showed elevation in the values of SGPT, 14 cases (28%) showed elevation in values of SGOT and 19 cases (38%) showed elevation in ALP values. There were 8 cases (16%), 4 cases (8%) and 8 cases (16%) having elevated values of TB, IB and DB respectively.

There were statistically non-significant (P ≤ 0.05) variations in biochemical parameters within different breeds **(Table 18)** but there was statistically significant variation found in the values of SGPT within different age group **(Table 19)**.

In renal insufficiency there is increase in the level of creatinine and BUN which might be due lesions were seen in glomeruli which results inreduction in the rate of glomerular filtration rate which invariably caused increase in the

values of BUN and creatinine as there is rate of production exceeds rate of excretion. Higher amount of BUN in circulation causes endothelial degeneration and hepatic degeneration which leads to elevation in values of SGPT and ALP. There is decrease in the values ofTP and ALB could be due to an increased filtration of protein and albumin through glomeruli owing to its molecular size and excretes through urine (Booth, 1990 and Shaw and Ihle 2013).

These results were in accordance with the findings of previous studies by Picut (1985), Macdougall *et al*., (1986), Benjamin (2001), DiBartola (2005) and Sharma and Shrestha (2011) they found that increase in creatinine and BUN level in renal failure dogs. Miyamoto *et al.* (1997), Robertson and Seguin (2008) and Kandula and Karlapudi (2015) had recorded elevation in creatinine and BUN level whereas, decrease in total protein and albumin in dogs with kidney failure.Adin and Cowgill (2000)observed average BUN concentration was 122 ± 71 mg/dL whereas the average Serum creatinine concentration was 7.5 ± 5.0 mg/dL in cases of acute renal failure and Koyner *et al*., (2010) found that AKI patient had higher serum creatinine. Cowgill (1992), Bradea, *et al*., (2013) and Oburai *et al.* (2015) found increase in the values of creatinine and BUN in dogs with CKD.

Table 13: Mean values of Biochemical parameters in clinical cases: (Group wise)

Parameters	Group of Animals		
	ARF	**CRF**	**Healthy**
SGPT (IU/L)	88.44 ± 9.19^{NS}	62.67 ± 7.9^{NS}	50.04 ± 4.76^{NS}
SGOT(IU/L)	73.373 ± 9.89^{NS}	42.96 ± 3.58^{NS}	41.27 ± 3.41^{NS}
ALP(IU/L)	358.55 ±121.07^{NS}	132.38 ± 15.30^{NS}	108.8± 29.27^{NS}
TP (g/dl)	6.36 ± 0.18^{NS}	6.59 ± 0.16^{NS}	6.42 ± 0.34^{NS}
ALB (g/dl)	2.58 ± 0.08^{NS}	2.55 ± 0.15^{NS}	2.61 ± 0.22^{NS}
GLB (g/dl)	3.76 ± 0.19^{NS}	3.94 ± 0.23^{NS}	3.81 ± 0.3^{NS}
TB (mg/dl)	1.43 ± 0.35^{NS}	0.56 ± 0.12^{NS}	0.62 ± 0.13^{NS}
DB (mg/dl)	0.75 ± 0.21^{NS}	0.26 ± 0.05^{NS}	0.28 ± 0.06^{NS}
IB (mg/dl)	0.67 ± 0.17^{NS}	0.29 ± 0.06^{NS}	0.34 ± 0.07^{NS}
BUN (mg/dl)	114.2 ±15.66^{a}	91.75 ± 22^{a}	20.46 ± 2.45^{a}
CREAT (mg/dl)	10.74 ± 0.96^{a}	4.42 ± 0.89^{b}	1.05 ± 0.13^{b}

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 14: Mean values of Biochemical parameters in clinical cases: (Age wise)

Parameters	Group of Animals		
	0-5 years	**6-10 years**	**11-15 years**
SGPT (IU/L)	100.81 ± 14.22^{a}	63.64 ± 7.07^{a}	92.48 ± 15.94^{a}
SGOT(IU/L)	73.63 ± 16.62^{NS}	50.9 ± 3.51^{NS}	82.883 ± 21.14^{NS}
ALP(IU/L)	543.21± 223.89^{NS}	154.35 ± 41.08^{NS}	153.382 ± 40.84^{NS}
TP (g/dl)	6.55 ± 0.24^{NS}	6.32 ± 0.18^{NS}	6.573 ± 0.42^{NS}
ALB (g/dl)	2.72 ± 0.1^{NS}	2.5 ± 0.1^{NS}	2.697 ± 0.26 ^{NS}
GLB (g/dl)	3.79 ± 0.28^{NS}	3.82 ± 0.19^{NS}	3.877 ± 0.41 ^{NS}
TB (mg/dl)	1.28 ± 0.46^{NS}	1.09 ± 0.36^{NS}	1.048 ± 0.3 ^{NS}
DB (mg/dl)	0.7 ± 0.19^{NS}	0.46 ± 0.25^{NS}	0.503 ± 0.24 ^{NS}
IB (mg/dl)	0.58 ± 0.28^{NS}	0.62 ± 0.13^{NS}	0.545 ± 0.79 ^{NS}
BUN (mg/dl)	92.56 ± 22.03^{NS}	101.41 ± 16.63^{NS}	165.032 ± 23.65 ^{NS}
CREAT (mg/dl)	8.32 ± 1.08^{NS}	7.98 ± 1.27^{NS}	12.618 ± 1.97^{NS}

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 15:Mean values of Biochemical parameters in clinical cases:(Breed wise)

Parameters	Breed		
	Labrador	German Shephard	Nondescript
SGPT (IU/L)	78.74 ± 10.97 NS	68.57 ± 4.86 NS	75.98 ± 19.61 NS
SGOT(IU/L)	68.83 ± 14.15 NS	43.5 ± 7.45 NS	62.04 ± 8.18 NS
ALP(IU/L)	266.92 ± 64.18 NS	91.35 ± 34.79 NS	671.52 ± 513.37 NS
TP (g/dl)	6.46 ± 0.23 NS	6.3 ± 0.57 NS	6.31 ± 0.25 NS
ALB (g/dl)	2.45 ± 0.1 NS	2.56 ± 0.2 NS	2.86 ± 0.15 NS
GLB (g/dl)	3.99 ± 0.25 NS	3.74 ± 0.37 NS	3.45 ± 0.37 NS
TB (mg/dl)	1.77 ± 0.57 NS	0.42 ± 0.01 NS	0.85 ± 0.23 NS
DB (mg/dl)	0.94 ± 0.34 NS	0.29 ± 0.23 NS	0.39 ± 0.11 NS
IB (mg/dl)	0.82 ± 0.27 NS	0.21 ± 0.01 NS	0.46 ± 0.12 NS
BUN (mg/dl)	88.19 ± 18.37 NS	187.15 ± 49.44 NS	99.52 ± 34.42 NS
CREAT (mg/dl)	8.66 ± 1.44 NS	10.92 ± 3.53 NS	8.05 ± 3.39 NS

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 16: Mean values of Biochemical parameters in clinical cases :(Breed wise)

Parameters	Breed	
	Pomeranian	Other breeds
SGPT (IU/L)	86.94 ± 14.33 NS	88.75 ± 18.92 NS
SGOT(IU/L)	82.02 ± 21.19 NS	45.91 ± 4.19 NS
ALP(IU/L)	126.15 ± 19.95 NS	281.84 ± 142.17 NS
TP (g/dl)	6.48 ± 0.26 NS	6.48 ± 0.33 NS
ALB (g/dl)	2.63 ± 0.13 NS	2.74 ± 0.24 NS
GLB (g/dl)	3.84 ± 0.28 NS	3.74 ± 0.35 NS
TB (mg/dl)	0.61 ± 0.04 NS	0.86 ± 0.23 NS
DB (mg/dl)	0.26 ± 0.02 NS	0.48 ± 0.18 NS
IB (mg/dl)	0.34 ± 0.45 NS	0.38 ± 0.49 NS
BUN (mg/dl)	116.78 ± 30.83 NS	106.66 ± 23.41 NS
CREAT (mg/dl)	10.62 ± 1.8 NS	6.87 ± 1.43 NS

Values (mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P ≤ 0.05).

Table 17: Analysis of variance of Biochemical parameters in clinical cases: (Group wise)

Parameters	Source of variation	Df	SS	MS	F cal	CD value at 5%
SGPT (IU/L)	Treatments	2	11156.820	5578.410	2.940	NS
	Error	47	89175.464	1897.350	-	
	Total	49	-	-	-	
SGOT (IU/L)	Treatments	2	10750.711	5375.355	2.773	NS
	Error	47	91096.459	1938.223	-	
	Total	49	-	-	-	
ALP (IU/L)	Treatments	2	656845.735	328422.868	1.163	NS
	Error	47	13269115.794	282321.613	-	
	Total	49	-	-	-	
TP (g/dl)	Treatments	2	0.513	0.257	0.287	NS
	Error	47	41.998	0.894	-	
	Total	49	-	-	-	
ALB (g/dl)	Treatments	2	0.053	0.026	0.096	NS
	Error	47	12.913	0.275	-	
	Total	49	-	-	-	
GLO(g/dl)	Treatments	2	0.283	0.141	0.142	NS
	Error	47	46.859	0.997	-	
	Total	49	-	-	-	
TB (mg/dl)	Treatments	2	8.698	4.349	1.714	NS
	Error	47	119.273	2.538	-	
	Total	49	-	-	-	
DB (mg/dl)	Treatments	2	2.834	1.417	1.561	NS
	Error	47	42.670	0.908	-	
	Total	49	-	-	-	
IB (mg/dl)	Treatments	2	1.626	0.813	1.409	NS
	Error	47	27.119	0.577	-	
	Total	49	-	-	-	
BUN (mg/dl)	Treatments	2	44411.016	22205.508	3.302	5.332*
	Error	47	316043.749	6724.335	-	
	Total	49	-	-	-	
CREAT (mg/dl)	Treatments	2	691.778	345.889	16.417	95.256*
	Error	47	990.215	21.068	-	
	Total	49	-	-	-	

NS Non significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

Table 18: Analysis of variance of Biochemical parameters in clinical cases: (Age wise)

Parameters	Source of variation	Df	SS	MS	F cal	CD value at 5%
SGPT (IU/L)	Treatments	2	13586.781	6793.391	3.434	51.873*
	Error	41	81112.086	1978.344	-	
	Total	43	-	-	-	
SGOT (IU/L)	Treatments	2	7431.346	3715.673	1.665	NS
	Error	41	91500.060	2231.709	-	
	Total	43	-	-	-	
ALP (IU/L)	Treatments	2	1496520.107	748260.053	2.508	NS
	Error	41	12231312.623	298324.698	-	
	Total	43	-	-	-	
TP (g/dl)	Treatments	2	0.586	0.293	0.320	NS
	Error	41	37.530	0.915	-	
	Total	43	-	-	-	
ALB (g/dl)	Treatments	2	0.528	0.264	1.014	NS
	Error	41	10.664	0.260	-	
	Total	43	-	-	-	
GLO(g/dl)	Treatments	2	0.028	0.014	0.013	NS
	Error	41	43.822	1.069	-	
	Total	43	-	-	-	
TB (mg/dl)	Treatments	2	0.414	0.207	0.068	NS
	Error	41	125.443	3.060	-	
	Total	43	-	-	-	
DB (mg/dl)	Treatments	2	0.550	0.275	0.407	NS
	Error	41	27.745	0.677	-	
	Total	43	-	-	-	
IB (mg/dl)	Treatments	2	0.042	0.021	0.019	NS
	Error	41	44.784	1.092	-	
	Total	43	-	-	-	
BUN (mg/dl)	Treatments	2	24050.586	12025.293	1.663	NS
	Error	41	296514.544	7232.062	-	
	Total	43	-	-	-	
CREAT (mg/dl)	Treatments	2	105.846	52.923	1.716	NS
	Error	41	1264.391	30.839	-	
	Total	43	-	-	-	

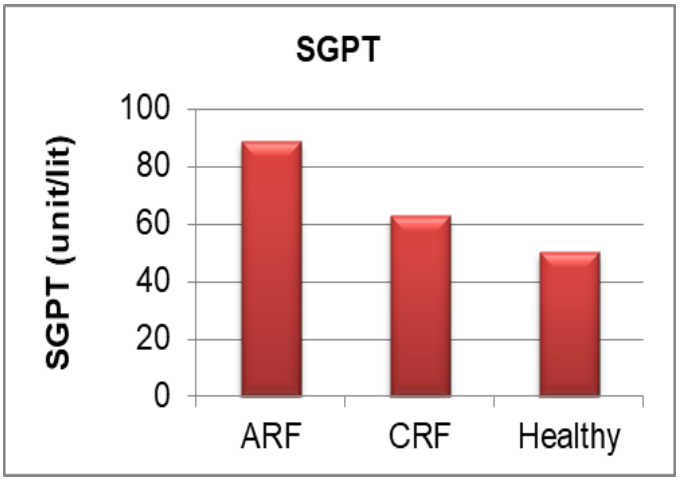
NSNon significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

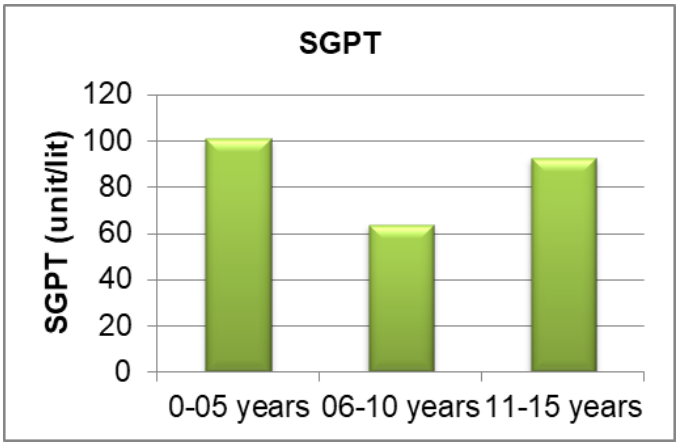
Table 19: Analysis of variance of Biochemical parameters in clinical cases: (Breed wise)

Parameters	Source of variation	Df	SS	MS	F cal	CD value at 5%
SGPT (IU/L)	Treatments	4	2365.006	591.251	0.250	NS
	Error	39	92333.862	2367.535	-	
	Total	43	-	-	-	
SGOT (IU/L)	Treatments	4	7299.732	1824.933	0.777	NS
	Error	39	91631.674	2349.530	-	
	Total	43	-	-	-	
ALP (IU/L)	Treatments	4	1228822.488	307205.622	0.959	NS
	Error	39	12499010.241	320487.442	-	
	Total	43	-	-	-	
TP (g/dl)	Treatments	4	0.220	0.055	0.057	NS
	Error	39	37.896	0.972	-	
	Total	43	-	-	-	
ALB (g/dl)	Treatments	4	1.002	0.251	0.959	NS
	Error	39	10.190	0.261	-	
	Total	43	-	-	-	
GLB(g/dl)	Treatments	4	1.430	0.358	0.329	NS
	Error	39	42.420	1.088	-	
	Total	43	-	-	-	
TB (mg/dl)	Treatments	4	12.289	3.072	1.055	NS
	Error	39	113.568	2.912	-	
	Total	43	-	-	-	
DB (mg/dl)	Treatments	4	3.939	0.985	0.939	NS
	Error	39	40.888	1.048	-	
	Total	43	-	-	-	
IB (mg/dl)	Treatments	4	2.348	0.587	0.882	NS
	Error	39	25.947	0.665	-	
	Total	43	-	-	-	
BUN (mg/dl)	Treatments	4	33070.689	8267.672	1.121	NS
	Error	39	287566.553	7373.501	-	
	Total	43	-	-	-	
CREAT (mg/dl)	Treatments	4	78.167	19.542	0.590	NS
	Error	39	1292.071	33.130	-	
	Total	43	-	-	-	

^{NS}Non significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

Figure 7: Mean values of SGPT in clinical cases: (Group wise, Age wise, Breed wise)





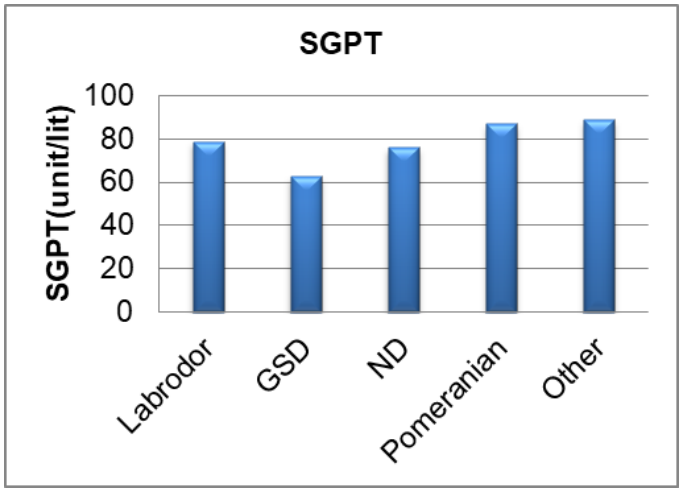
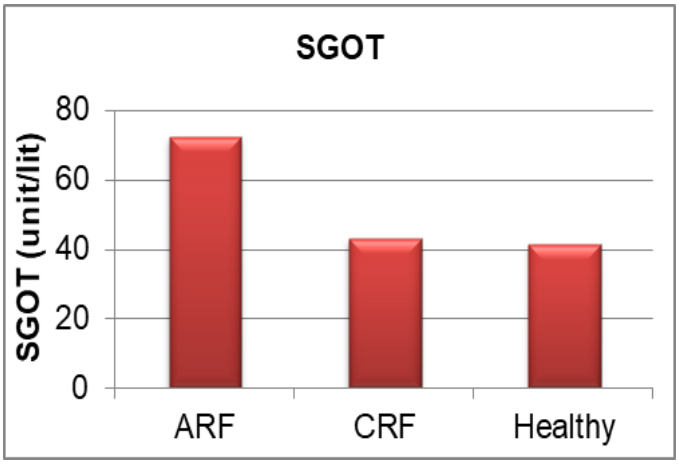
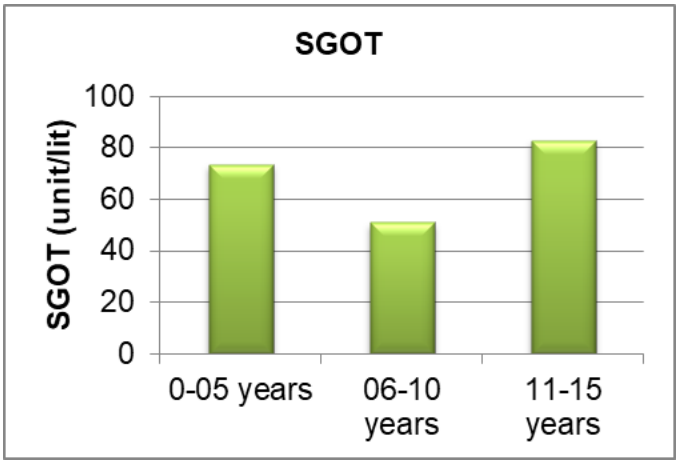


Figure 8: Mean values of SGOT in clinical cases: (Group wise, Age wise, Breed wise)





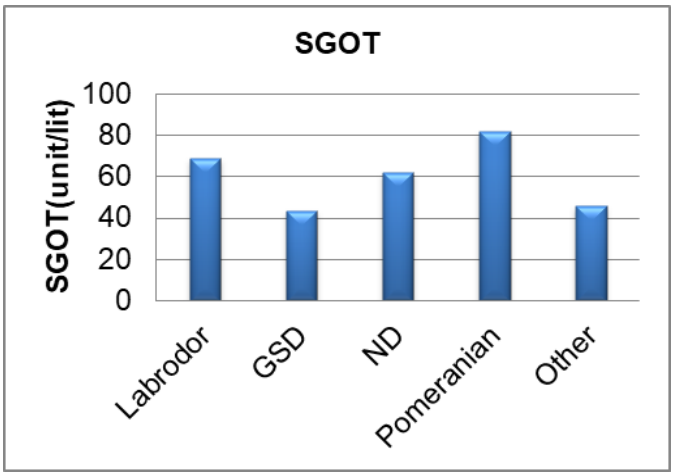
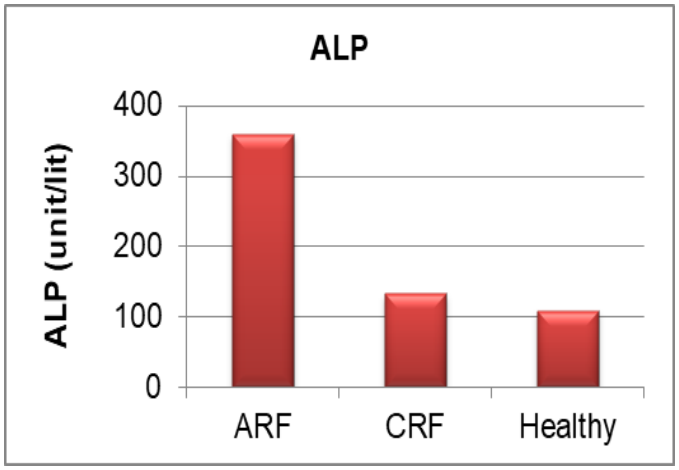
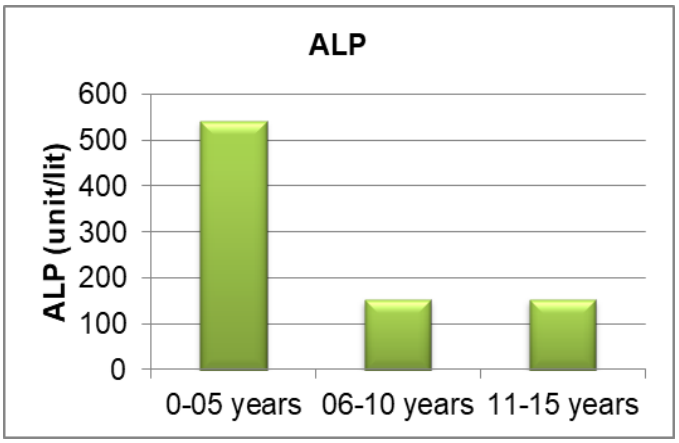


Figure 9: Mean values of ALP in clinical cases: (Group wise, Age wise, Breed wise)





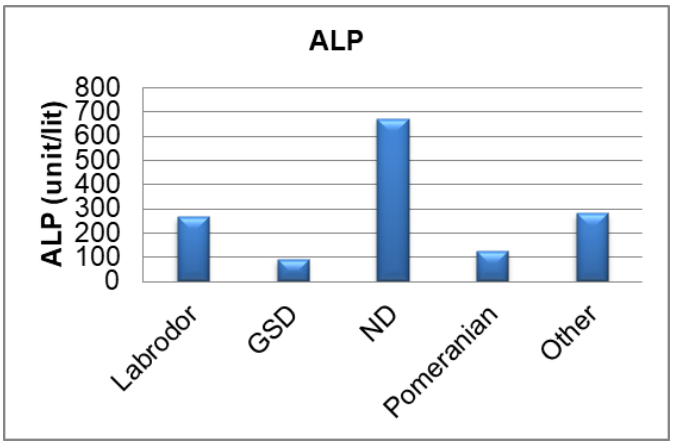
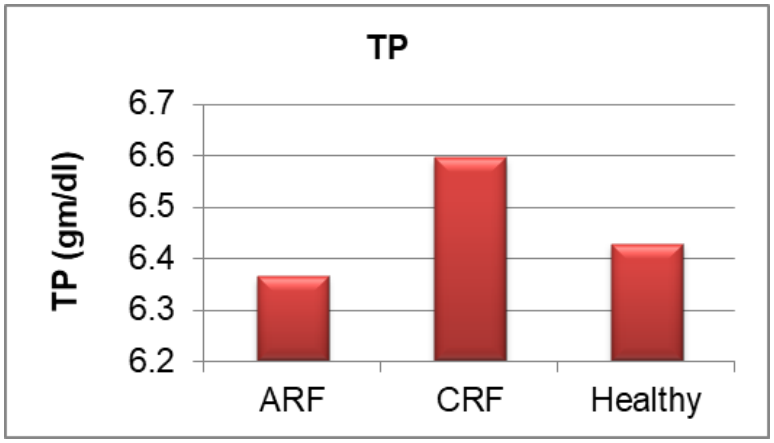
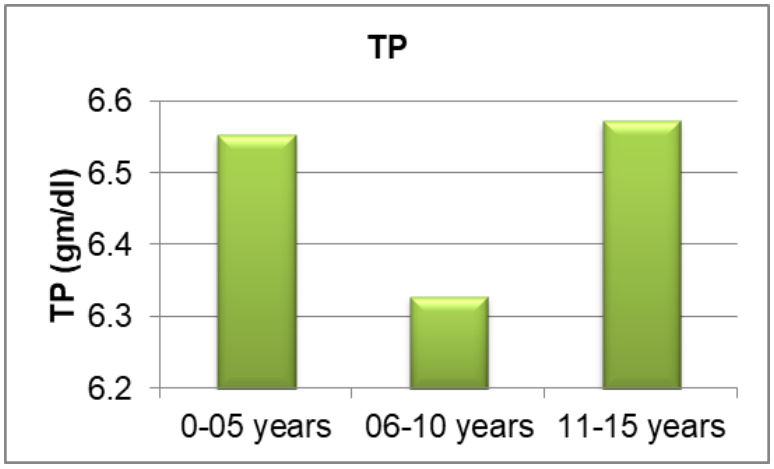


Figure 10: Mean values of TP in clinical cases: (Group wise, Age wise, Breed wise)





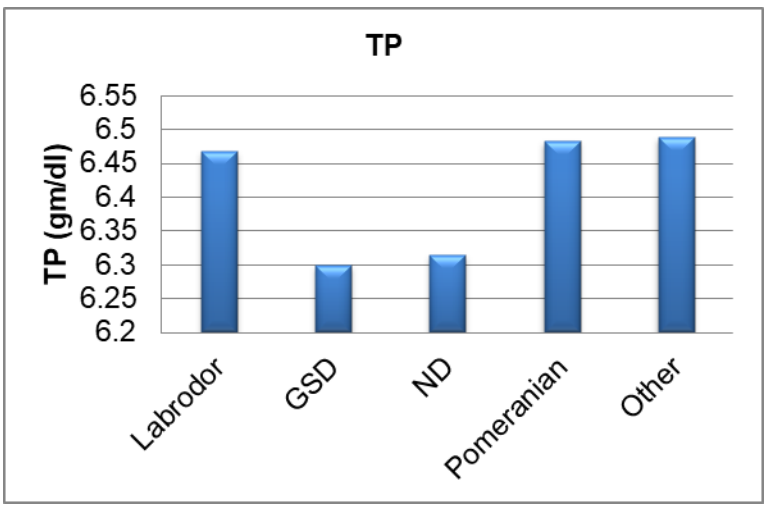
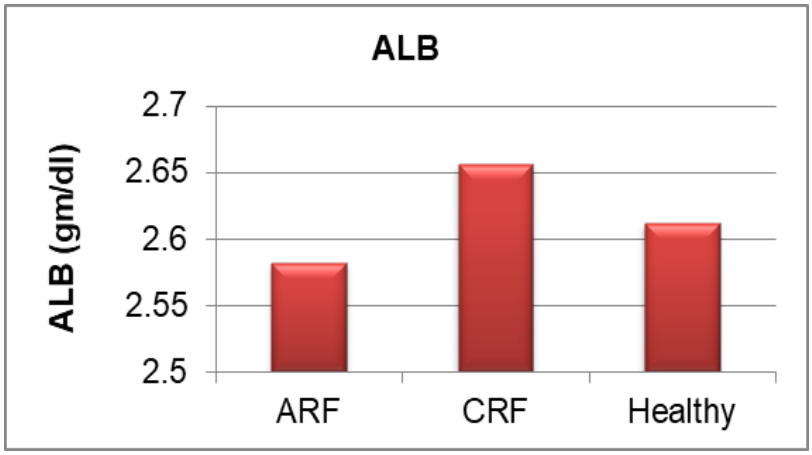
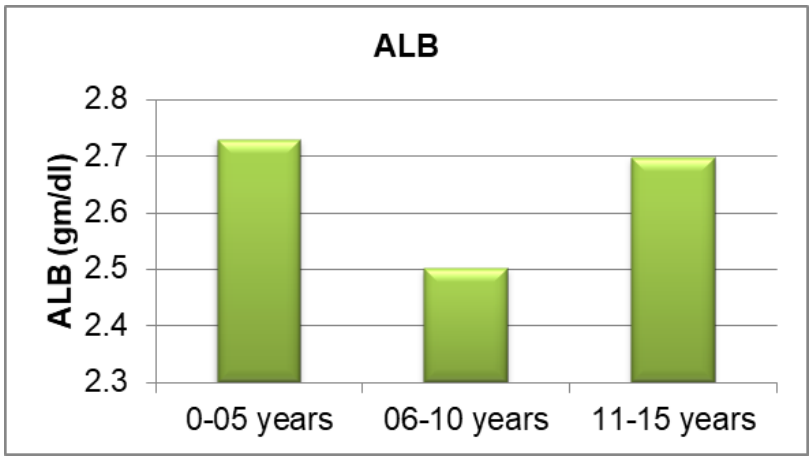


Figure 11: Mean values of ALB in clinical cases: (Group wise, Age wise, Breed wise)





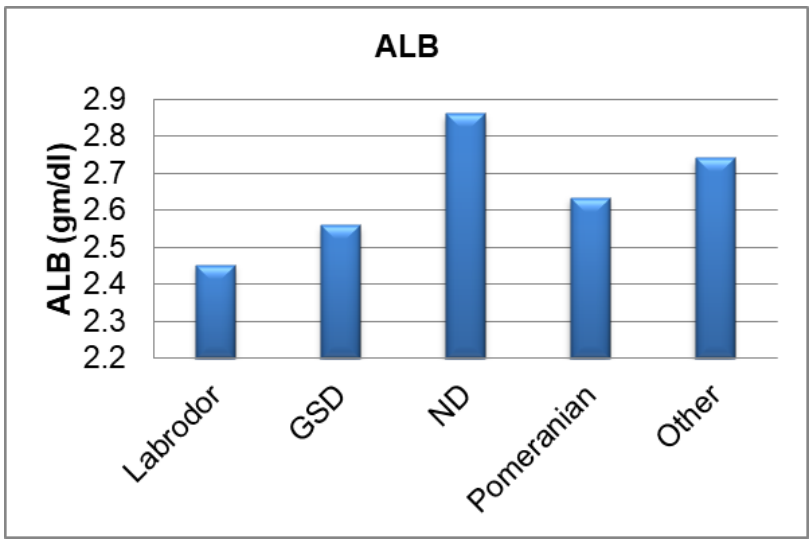
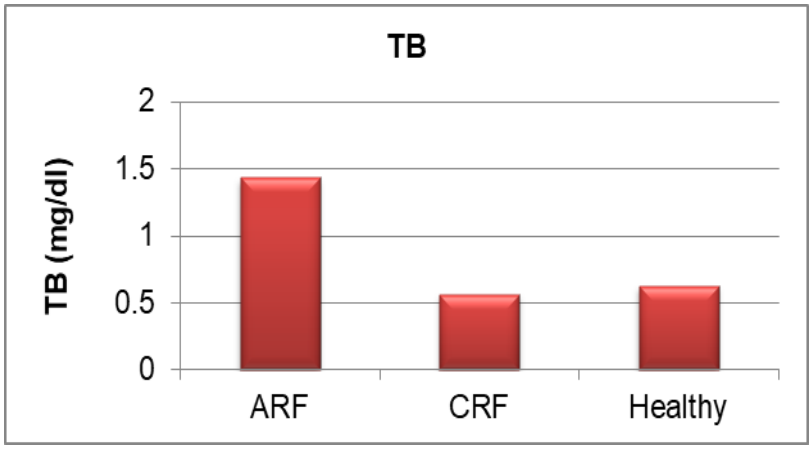
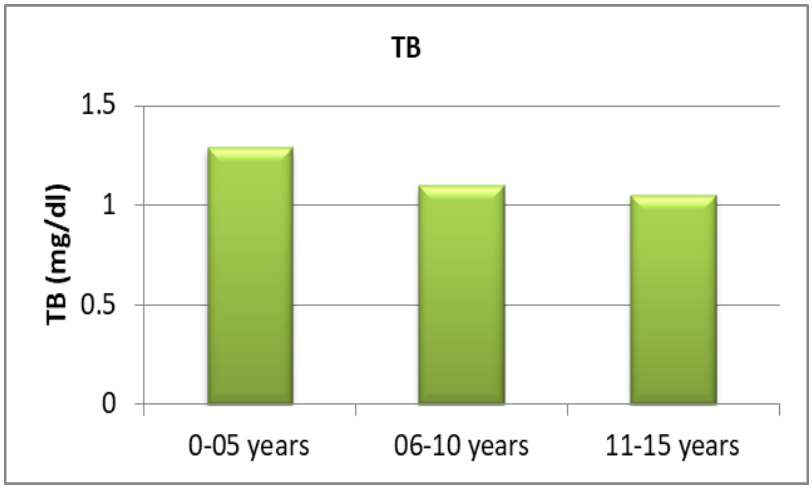


Figure 12: Mean values of TB in clinical cases: (Group wise, Age wise, Breed wise)





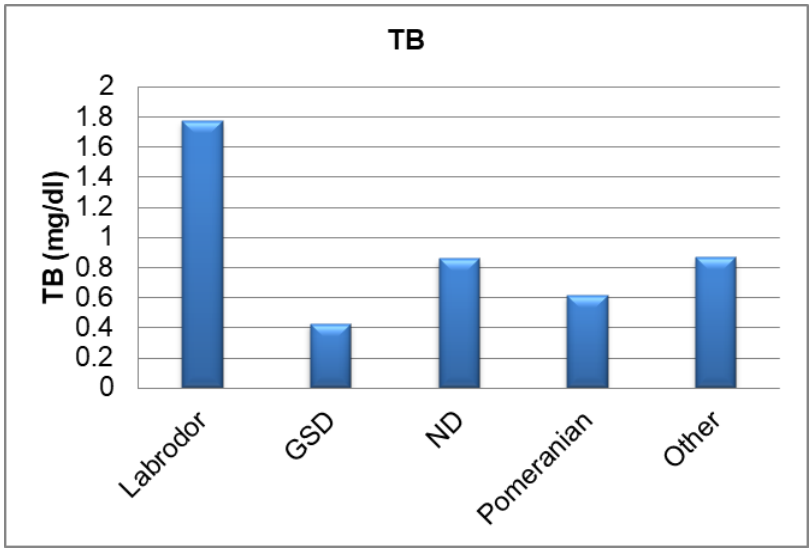
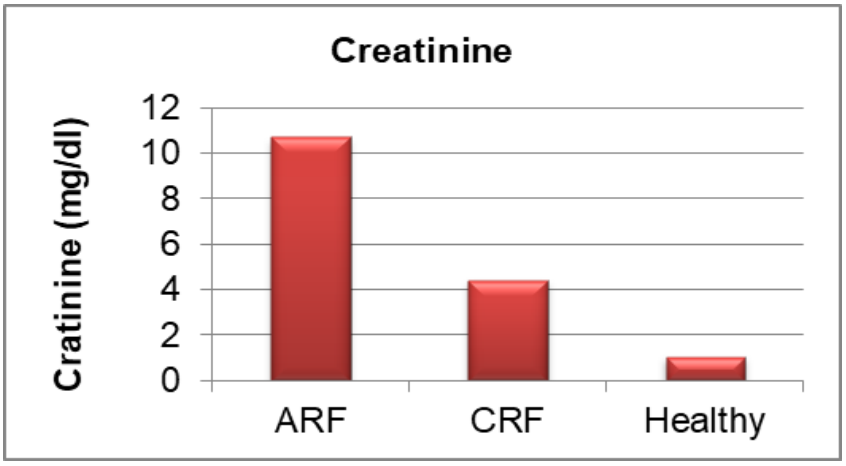
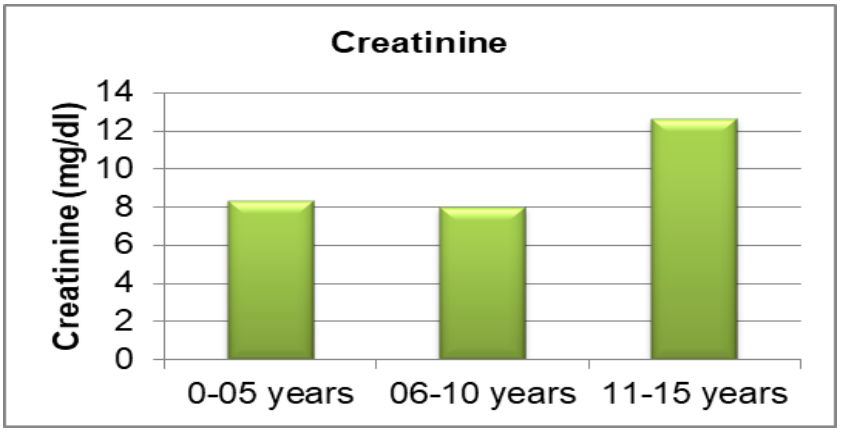


Figure 13: Mean values of creatinine in clinical cases: (Group wise, Age wise, Breed wise)





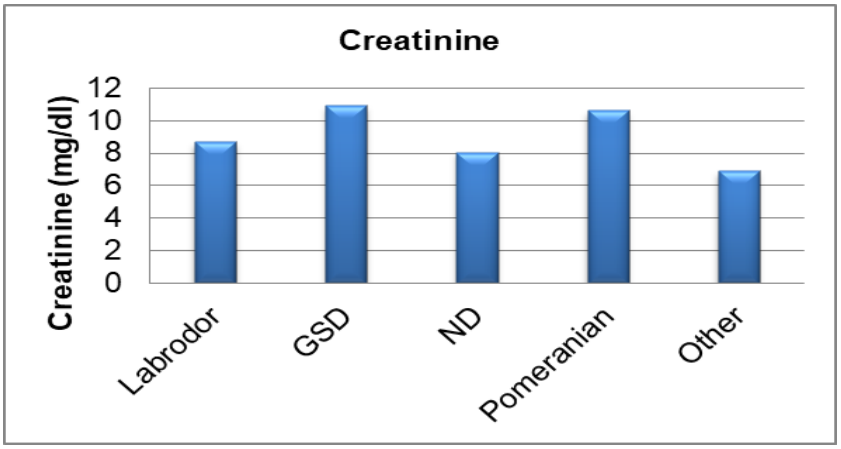
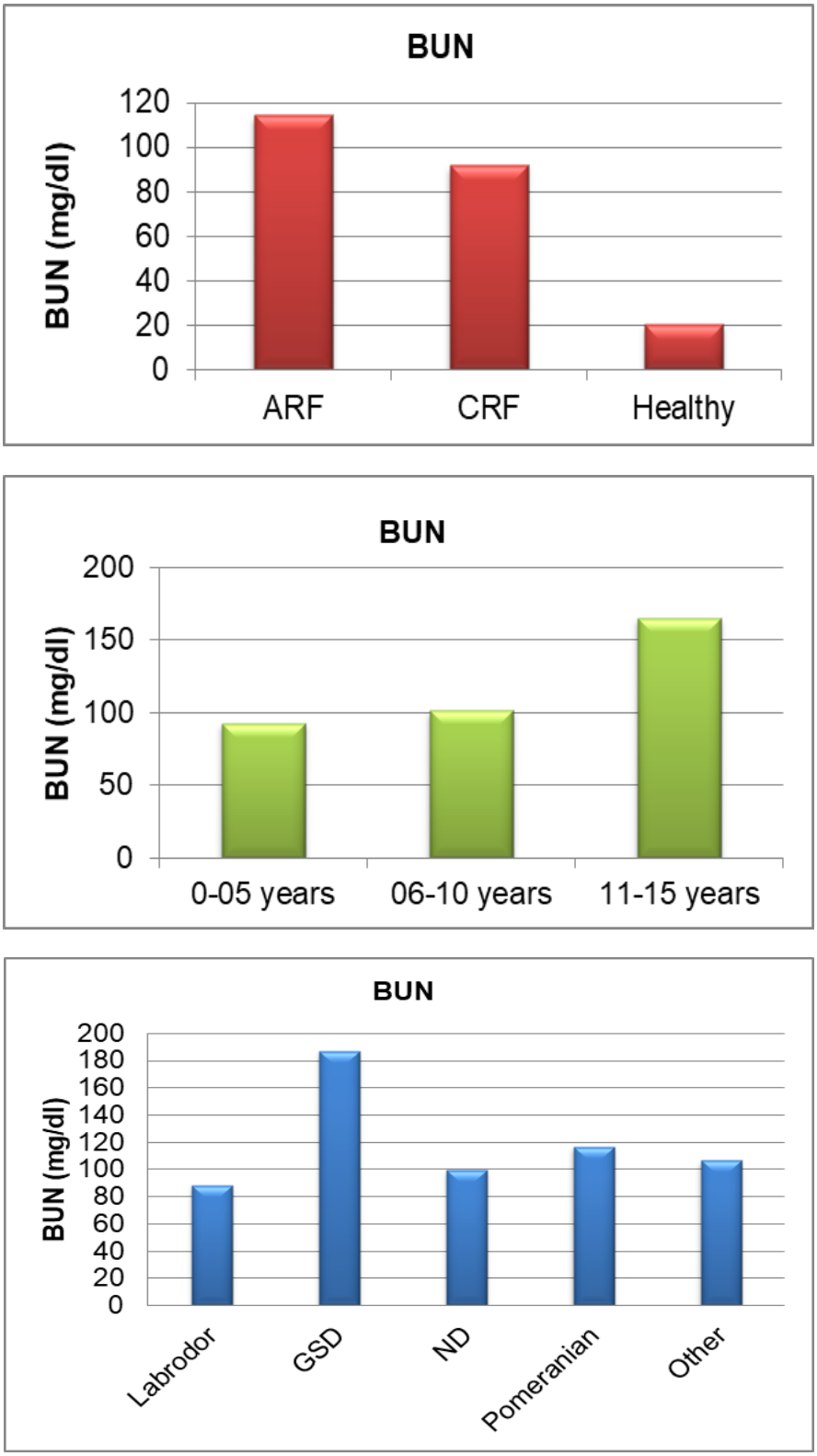


Figure 14: Mean values of BUN in clinical cases: (Group wise, Age wise, Breed wise)



4.4 Urinalysis

The Summary of the findings of urinalysis in clinical cases was given in **Table 20.** In the present study, all clinical cases were positive for protein in chemical examination of urine except 6 healthy cases which have taken as control. All samples were ammonical in odour. Color varied from faint yellow to dark yellow to brown and pH varied from 4.5 to 5.5. Microscopic examination of urine samples revealed uric acid and triple phosphate crystals in some cases. This is in agreement with the findings of Brown *et al*., (1985), Picut and Lewis (1987[b]), Robertson and Seguin (2008), Harley and Langston (2012) and Oburai *et al.* (2015).

Any small proteins that pass through a healthy glomerulus are reabsorbed by the renal tubules whereas, persistent proteinuria, is usually an indication of renal damage or dysfunction and less commonly persistent proteinuria can be caused by increased circulating levels of low molecular weight proteins (Harley and Langston, 2012).

Table 20: Summary of Urinalysis of urine samples collected from clinical cases

Parameters Case No.	Protein	Colour	Odor	pH	Transparency
1	Present	Pale Yellow	Ammonical	5.0	Cloudy
2	Present	Yellow	Ammonical	6.5	Cloudy
3	Present	Yellow	Ammonical	6.5	Cloudy
4	Present	Brown	Ammonical	5.0	Cloudy
5	Present	Yellowish brown	Ammonical	7.0	Cloudy
6	Present	Pale yellow	Ammonical	5.5	Hazy
7	Present	Yellow	Ammonical	5.5	Clear
8	Present	Pale Yellow	Ammonical	5.5	Hazy
9	Present	Brownish Yellow	Ammonical	6.0	Clear
10	Present	Faint yellow	Ammonical	5.0	Slightly cloudy
11	Present	Faint yellow	Ammonical	4.5	Cloudy
12	Present	Yellow	Ammonical	5.5	Clear
13	Present	Faint yellow	Ammonical	6	Clear
14	Present	Yellow	Ammonical	6.5	Cloudy
15	Present	Faint yellow	Ammonical	6.5	Clear
16	Present	Faint yellow	Ammonical	5.0	Cloudy
17	Present	Yellow	Ammonical	4.5	Clear
18	Present	Dark Yellow	Ammonical	6.0	Cloudy
19	Present	Brown	Ammonical	6.0	Cloudy
20	Present	Yellow	Ammonical	6.5	Clear
21	present	Dark yellow	Ammonical	4.5	Clear
22	Present	Brown	Ammonical	5.0	Clear
23	Present	Yellow	Ammonical	6.5	Cloudy
24	Present	Pale	Ammonical	6.0	Cloudy
25	present	Yellow	Ammonical	5.5	Clear

...Summary of Urinalysis

Parameters / Case No.	Protein	Colour	odor	pH	Transparency
26	Present	Faint Yellow	Ammonical	5.0	Clear
27	Present	Yellow	Ammonical	5.5	Cloudy
28	Present	Dark Yellow	Ammonical	5.5	Clear
29	Present	Yellow	Ammonical	6.0	Cloudy
30	Present	Dark Yellow	Ammonical	6.0	Cloudy
31	Present	Faint yellow	Ammonical	6.0	Clear
32	Present	Yellow	Ammonical	6.5	Clear
33	Present	Faint yellow	Ammonical	4.5	Cloudy
34	Present	Brownish yellow	Ammonical	6.0	Hazy
35	Present	Pale Yellow	Ammonical	6.0	Hazy
36	Present	Yellow	Ammonical	6.5	Clear
37	Present	Brown	Ammonical	4.5	Clear
38	Present	Yellowish brown	Ammonical	5	Clear
39	Present	Faint yellow	Ammonical	5.5	Clear
40	Present	Yellow	Ammonical	4.5	Cloudy
41	Present	Dark Yellow	Ammonical	7.0	Hazy
42	Present	Yellow	Ammonical	6.5	Cloudy
43	Present	Reddish brown	Ammonical	6.5	Turbid
44	Present	Brown	Ammonical	5.0	Clear
45	Absent	Yellow	Ammonical	5.5	Clear
46	Absent	Dark yellow	Ammonical	6.5	Cloudy
47	Absent	Dark yellow	Ammonical	4.0	Clear
48	Absent	Yellow	Ammonical	4.5	Clear
49	Absent	Yellow	Ammonical	5.0	Cloudy
50	Absent	Brown	Ammonical	6.5	Clear

4.5 Estimation of renal biomarkers in canine renal disorder

4.5.1 Estimation of lipocalin (NGAL):

The mean of NGAL in present study was 187.76 ± 13.2, 117.71 ± 17.37 and 53.33 ± 7.08 for ARF, CRF and Healthy groups respectively.

Fifty dogs were included in the study. Six dogs (3 males and 3 females) were classified as healthy. Fourteen dogs (10 males and 4 females) were diagnosed with chronic renal failure. Thirty dogs (18 males and 12 females) were diagnosed with acute renal failure.

Samples were processed by ELISA (BIOPORTO). A standard curve for NGAL was obtained by using 8 calibrators (ranging from 0 to 400pg/ml) of canine NGAL reference standard. The concentrations of NGAL in clinical samples were calculated from a standard curve **(Plate 2)**.

The age of all dogs was in the range of 0.7 to 15 years. Compared with the dogs in the healthy group, the azotemic dogs were older and had higher serum creatinine and serum NGAL.

For all fifty cases, serum creatinine was significantly correlated with serum NGAL concentration and number of white blood cell count was also associated with serum NGAL concentration.

There was statistically significant difference in the values of NGAL within the groups ($P \leq 0.05$) **(Table 21)** and **(Figure 15 & 16)**. Dogs with AKI had the highest serum NGAL concentration when compared with CKD and healthy groups, whereas dogs with CKD had higher concentration of serum NGAL than that of healthy group.

These findings were in accordance with the findings of previous studies of Lee *et al.,* (2012) who recorded the NGAL level in the serum of seven dogs with AKI was significantly increased and this difference was sustained to 72 hours but

it increases quickly in urine as compare to serum. Ahn and Hyun (2013) they found that the median serum NGAL concentration in control dogs was lower than dogs with CKD. Hsu *et al.,* (2014[a]) found serum and urine NGAL concentrations in azotemic dogs were significantly higher than in nonazotemic dogs. Hsu *et al.,* (2014[b]) observed that the uNGAL concentrations in dogs with renal diseases were higher than dogs of control group. Steinbach *et al*., (2014) found plasma NGAL concentration was significantly higher in dogs with AKI compared with dogs with CKD (P = .027) and lower in healthy dogs as compare to AKI and CKD.

Table 21: Mean values of NGAL in clinical cases: (Group wise, age wise and Breed wise)

Parameter	**Group of Animals**		
Group wise	**ARF**	**CRF**	**Healthy**
NGAL (pg/ml)	187.76 ± 13.20[a]	117.71 ± 17.37[a]	53.33 ± 7.08[b]
Age wise	**0-5 Years**	**6-10 Years**	**11-15 Years**
NGAL (pg/ml)	165.5 ± 22.17[NS]	179.222 ± 13.85 [NS]	76.5 ± 35.38 [NS]
Breed wise	**Labrador**	**German Shephard**	**Nondescript**
NGAL (pg/ml)	190.857 ± 16.22 [N]	162 ± 41.01 [NS]	142.667 ± 15.49 [NS]
	Pomeranian	**Other breeds**	
	122.5 ± 27.67 [NS]	196 ± 36.43 [NS]	

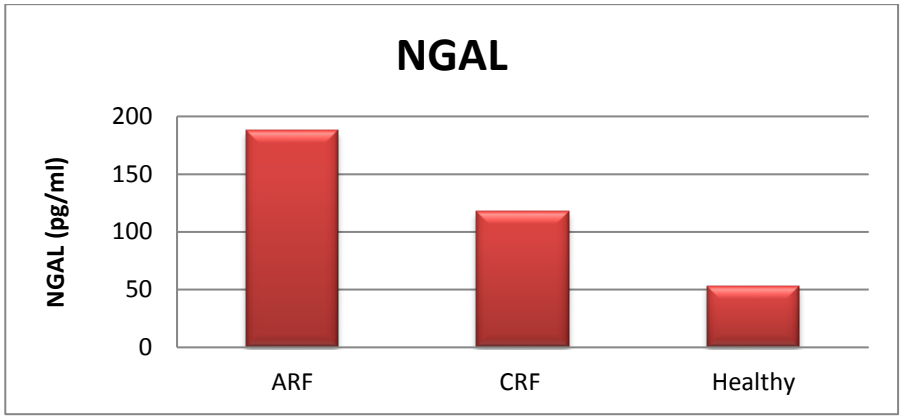
Values (mean ± SE) in the same row bearing at least one common superscript do not vary significantly (P ≤ 0.05).

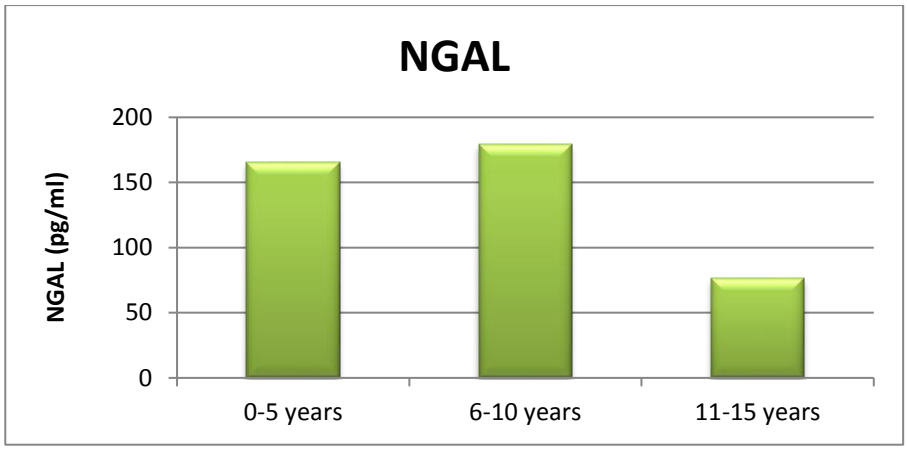
Table 22: Analysis of variance of serum NGAL in clinical cases:(Group wise, Age wise and Breed wise)

Parameters	**Source of variation**	**DF**	**SS**	**MS**	**F cal**	**CD value at 5%**
Group wise	Treatments	2	98507.520	49253.760	13.729	70.199*
NGAL (pg/ml)	Error	35	125563.322	3587.523	-	
	Total	37	-	-	-	
Age wise	Treatments	2	34723.948	17361.974	3.132	NS
NGAL (pg/ml)	Error	31	171837.111	5543.133	-	
	Total	33	-	-	-	
Breed wise	Treatments	4	23591.827	5897.957	1.254	NS
NGAL (pg/ml)	Error	27	126998.048	4703.631	-	
	Total	31	-	-	-	

[NS]Non significant * Significant at P≤ 0.05 ** Significant at P ≤ 0.01

Figure 15: Mean values of NGAL in clinical cases: (Group wise, Age wise, Breed wise)





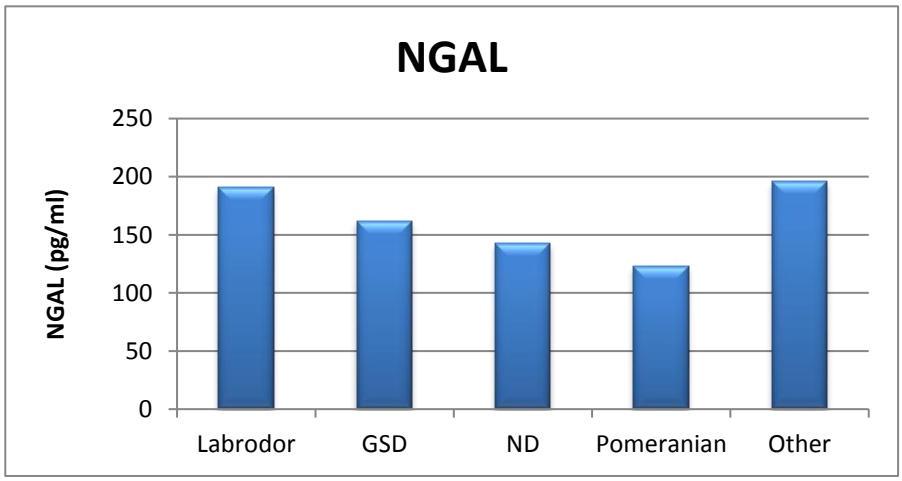
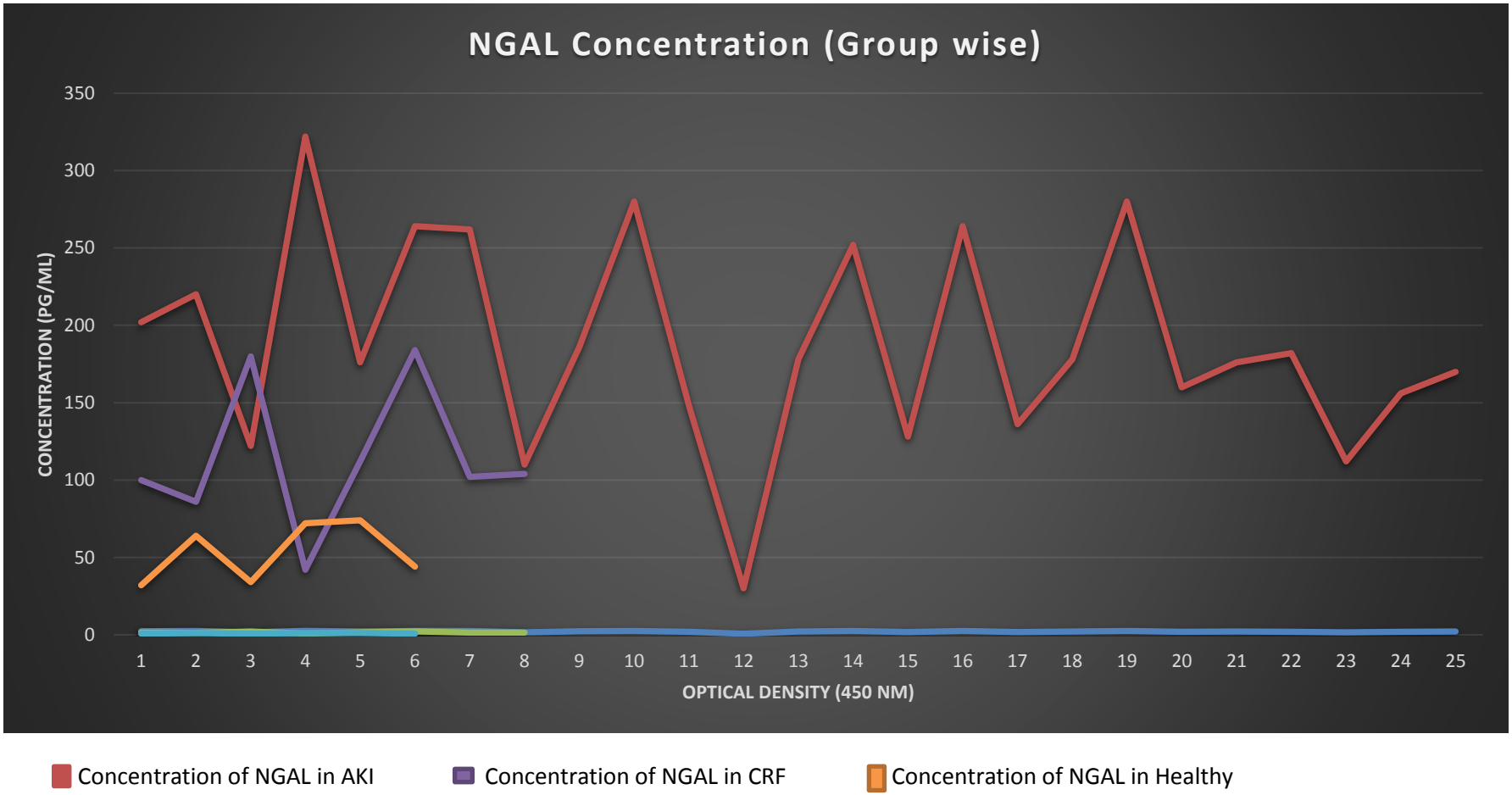


Figure 16: Graphical comparison of NGAL concentration between the groups



4.5.2 Estimation of Beta 2 microglobulin (B2M):

In this estimation amongst these 50 samples, 14 CRF samples were included. This includes 10 males and 4 females.

These samples were processed by ELFA (mini VIDAS) for presence of amyloidosis in kidney. The result for B2M was negative as all the 14 values were in normal range (0.3 to 3.0 mg/l).

As all the samples were in normal range, it indicates there was no deposition of amyloid in kidneys of these 14 cases (Ahuja, *et al* 2010)

4.6 Gross observations of renal disorders in dog

Among 50 cases of renal dysfunction 23 dogs have died (46%; ARF(73.91) and CRF (26.09%)). Necropsy examination of 3 cases was done (Case no. 9, 31 and 34). Gross observations of kidneys and liver of necropsy cases revealed following information

In case no.9, kidneys were of normal size or slightly enlarged. Capsule peeled off easily. Grey areas were present in the outer medulla as well **(Plate 3)**

In case no. 34, kidneys were larger in size and hemorrhagic patches were seen on the surface of kidney and cystic cavity were present at the corticomedullary junction **(Plate 4 A, B)**.

In case no. 31, kidneys were of smaller in size and contracted, capsule was hard to peeled off, cortex was shrunken. The surface was uneven due to irregular contraction of the fibrous tissue **(Plate 5 A, B)**.

Apart from clinical cases, departmental necropsy cases were included in histopathological study to check renal lesions.

Gross observations of the kidneys of 25 necropsy cases revealed following information

In some cases, kidneys were of normal size or slightly enlarged. Capsule peeled off easily. Hemorrhagic patches and congestion were present on surface of kidney **(Plate 7 A, B; 9 A, B).**

In 3 cases, kidneys were of normal size, soft and pulpy in consistency and capsule peeled off easily.

In six cases, kidneys were normal in size and had white necrotic foci on the cortical surface of kidney **(Plate 6and 8)**.

In most of the cases, kidneys were smaller in size, pale to grey in color, were hard to cut and capsule was very hard to peeled off. The cortex was shrunken. The surface was uneven due to irregular contraction of the fibrous tissue.

These gross findings were in agreement with Ganti and Rao (1968) who observed in interstitial nephritis in the acute type, the kidney may be of normal size or slightly enlarged, capsule strips off easily. In subacute and chronic types of interstitial nephritis kidneys were smaller in size, pale to grey in color, was hard to cut and capsule peeled off with difficulty. The surface was uneven due to irregular contraction of the fibrous tissue “Small granular contracted kidney”. In glomerulonephritis in acute type, both the kidneys were enlarged and pale, capsule peeled off easily. In sub-acute type, kidneys were enlarged, pale and smooth with non-adherent capsule. In chronic type, kidneys were shrunken and contracted with finely granular surface

In case no.9, liver was congested and enlarged with rounding of borders. Multifocal white patches were found on surface of liver **(Plate 11)**. In case no. 34, liver was pale, firm and showed diffuse necrotic foci on all lobes of liver **(Plate 12 A, B)**. In case no. 31, liver was enlarged and pale in color.

These findings were associated Chandrasekaran *et al.,* (2011) observed Liver were enlarged with varying degrees of vascular & degenerative changes.

4.7 Histopathological examination of kidneys

Histopathological alterations were studied in the kidney and liver of clinical cases presented with renal failure by staining the sections of these tissues with hematoxylin and eosin stains as per standard procedure.

In case no. 9 cystic dilatation of tubules, multifocal tubular degeneration and coagulative necrosis and protein casts in tubular lumen was observed **(Plate 13 A, B).**

In case no. 31 there was focal glomerular atrophy, diffuse tubular degeneration and multifocal foci of coagulative necrosis in tubules. There was infiltration of mononuclear cells (predominantly plasma cells) and proliferation of fibrous connective tissue **(Plate 14)**.

In case no. 34 dilatation of Bowman's capsule and variation in the size of glomerulus. Tubules showed degeneration and necrosis. Infiltration of mononuclear cells was observed **(Plate 15 A, B, C)**.

In case no. 9, there was degenerative changes and congestion in liver **(Plate 30)**. In case no. 34, there was presence of atrophy of chords, degeneration and sinusoidal dilatation **(Plate 31)** and in case no. 31, there was degenerative changes in liver.

Apart from clinical cases, 25 departmental necropsy cases were included in histopathological study to check renal lesions.

The findings of 25 departmental necropsy cases were given in the following table **(Table 21)** and **(Plate 16 to Plate 29)**.

Table 23: Histopathological findings of departmental necropsy cases

Case No.	Glomerular Findings	Tubular Findings	Interstitial Findings
2	Focal glomerular atrophy (+) Diffuse increase in cellularity of glomerulus (++)	Diffuse tubular degeneration (+++) multifocal foci of coagulative necrosis (++)	Multifocal foci of infiltration of MNC (lymphocytes and plasma cells) (++)
3	Diffuse glomerular atrophy (++) Pinkish material in Bowman's capsule (BC) Hyalinization (+)	Diffuse tubular degeneration (+++) and coagulative necrosis (++)	Infiltration of MNC (lymphocytes) (+) Proliferation of fibrous tissue (+)
4	Hyalinization (+)	Tubular degeneration and necrosis (+)	Infiltration of MNC (+) Fibrosis (++)
5	----	Multifocal tubular degeneration (++)	Infiltration of MNC (++)
6	Atrophic glomeruli (+)	Diffuse dilatation of tubule (++) Accumulation of pinkish material in the tubules (++)	Congestion (++)
8	Multifocal areas of glomerular atrophy (+)	Multifocal are of tubular degeneration (++)	Multifocal areas of infiltration of MNC (+++)
12	Glomerular adhesion with BC (++) Mild increase in cellularity (+) Hyalinization of glomerulus (+)	Diffuse cellular swelling and coagulative necrosis in tubules (+)	Multifocal areas of congestion and hemorrhages (+)

13	Diffuse hyalinization of glomerulus (++) Diffuse increase in thickness of BC (++) Fibrosis around glomerulus (+)	Diffuse tubular degeneration (+) Coagulative necrosis (+)	Infiltration of MNC (+)
14	Multifocal increase in glomerular cellularity (++)	Multifocal degenerative and necrotic changes in tubules (+)	Multifocal infiltration of MNC, predominantly plasma cells (++)
16	Multifocal atrophy of glomerulus (+)	Multifocal tubular degeneration (++)	Multifocal infiltration of MNC (++) Focal proliferation of fibrous tissue (+)
20	Proliferation of fibrous tissue around glomerulus (+++) Thickening of BC (++)	Multifocal cystic dilatation of tubules (++) Multifocal tubular degeneration (+)	Multifocal infiltration of MNC (++) Multifocal proliferation of fibrous tissue (+)
22	Multifocal atrophy of glomerulus (+)	Multifocal tubular degeneration (+++)	Multifocal areas of fibrosis (++)
24	Diffuse fibrosis of BC (+) Accumulation of pinkish material in BC (+)	Diffuse tubular degeneration (+)	Multifocal areas of intense infiltration of MNC (lymphocytes and plasma cells) (+++)
26	Multifocal areas of atrophied glomeruli (+) Accumulation of proteinaseous fluid (+)	Diffuse tubular degeneration (+++)	Diffuse intense infiltration of MNC (+++)
33	Multifocal areas of atrophied glomeruli (++) Calcification of wall of BC (+)	Multifocal tubular degeneration and necrosis (++) Multifocaltubular wall calcification (++)	Proliferation of fibrous tissue (++)

36	----	Multifocal areas of coagulative necrosis of tubules (++)	----
30	Focal minimal glomerular atrophy (+) Increased cellularity (+)	Multifocal tubular degeneration and necrosis (++)	Multifocal areas of Infiltration of inflammatory cells in interstitium of MNC predominantly Plasma cells (++)
71/18	Increased cellularity of glomerulus (+)	Multifocal tubular degeneration and necrosis (++)	Multifocal Infiltration of inflammatory cells MNC predominantly Plasma cells (++)
86/18	Focal minimal glomerular atrophy (+)	Multifocal tubular degeneration (++) Tubular necrosis (+)	Multifocal areas of Infiltration of inflammatory cells in interstitium of MNC (+)
83	Increased cellularity of glomerulus (+) Atrophy (++)	Multifocal tubular degeneration (++) Tubular necrosis (+)	Diffuse Infiltration of inflammatory cells in interstitium MNC predominantly Plasma cells (++)
85/18	Hyalinization of glomerulus (++) Multifocal glomerulopathy (++)	Focal tubular dilatation (+)	Thickening of capsule (+) Diffuse infiltration of MNC beneath capsule and interstitium (++) Diffuse proliferation of fibrous connective tissue (++)
40	----	Diffuse tubular degeneration (++)	----
38	----	Diffuse tubular degeneration (++)	----
23	----	Diffuse tubular necrosis (+)	Congestion (+)

Summary of histopatholgical findings:

In total 27 necropsy cases, Congestion was found in 3 cases (11.11%) and hemorrhages were found in 1 case (3.7%). Degeneration and necrosis was found 21 cases (77.8%) and 16 cases (59.6%) respectively. Hyalinization of glomerulus were present in 5 cases (18.5%). Proliferation of fibrous tissue was present in 11 cases (40.74%) (Glomerulus: -3 cases and Interstitial tissue: - 8 cases).

These histopathological findings were associate with previous studies of Wright *et al.,* (1976)observed that diffuse renal fibrosis with chronic renal failure has been attributed mainly to chronic interstitial nephritis. Chew *et al.,* (1983)reported that the kidneys were characterized pathologically by glomerular sclerosis, cystic glomerular atrophy, tubular dilatation, tubular atrophy, mononuclear interstitial inflammation, interstitial fibrosis and interstitial mineralization. Ganti and Rao (2006) reported that in acute interstitial nephritis, infiltration of lymphocytes and plasma cells, with less number of neutrophils in interstitial tissue. In chronic type, there was cystic dilatation of tubules with hyaline casts. In acute glomerulonephritis, there is increased cellularity of the glomeruli, in sub-acute type, proliferation of epithelial cells of the parietal layer of the bowman's capsule results in epithelial crescents whereas, in chronic type, fibrosed glomeruli and disappearance of tubules.

These histopathological findings were in agreement with Eubig *et al.,* (2005) they observed congestion and degenerative changes in liver and Chandrasekaran *et al.,* (2011) recorded varying degrees of vascular and degenerative changes.

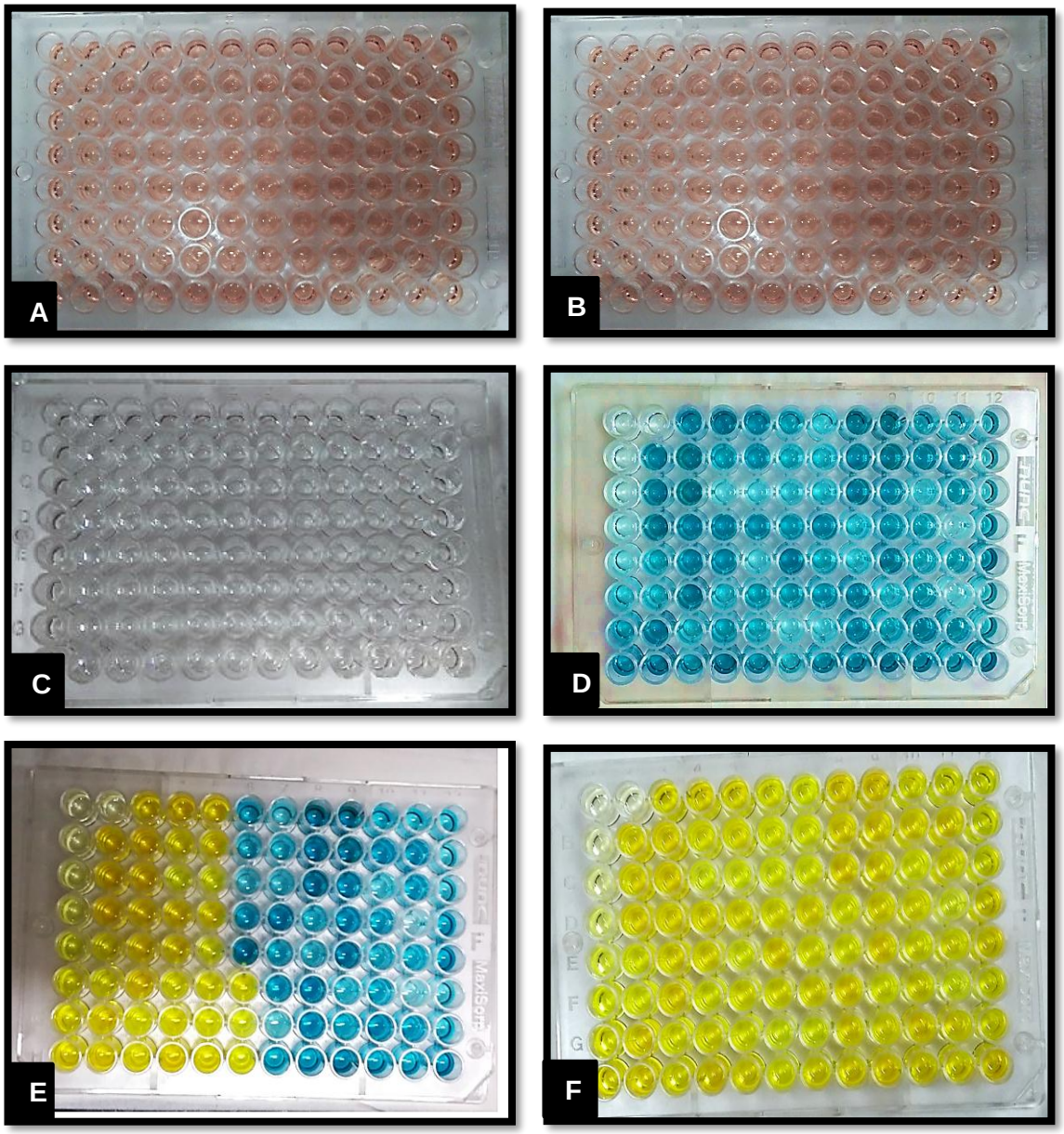


Plate 1: ELISA procedure for lipocalin;

A) Step 1: - Dog NGAL Calibrator and serum samples in ELISA plate,

B) Step 2: - Biotinylated Dog NGAL Antibody in ELISA plate,

C) Step 3: - HRP streptavidin in ELISA plate,

D) Step 4: - TMB substrate in ELISA plate,

E) Step 5: - Stop solution in wells which changes blue to yellow,

F) Final results

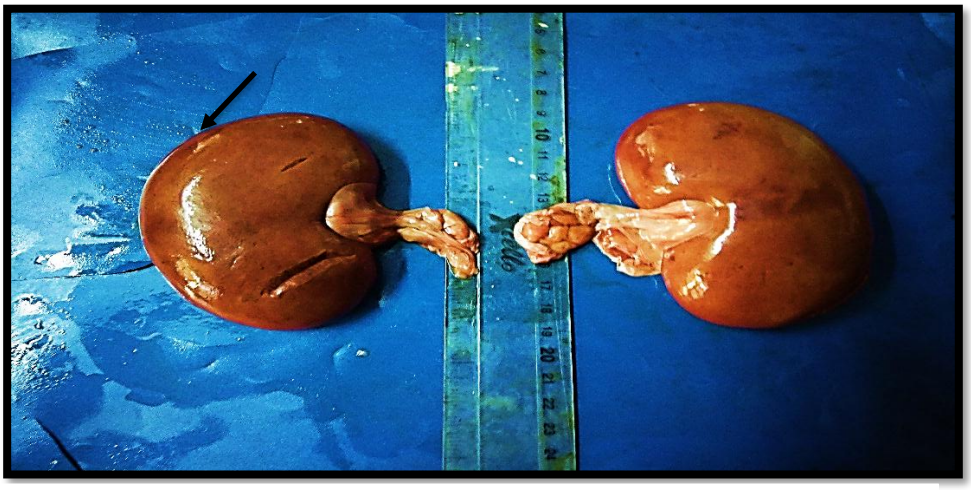
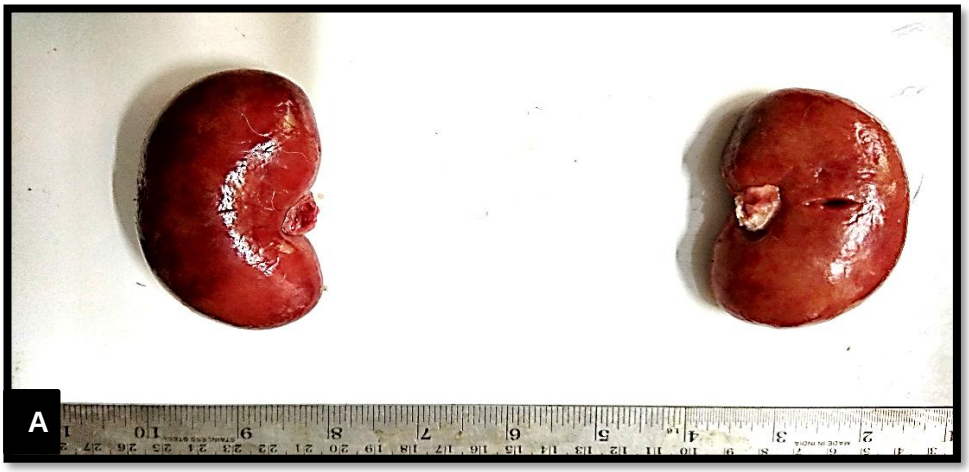


Plate 3: Necrotic foci on the surface of kidney



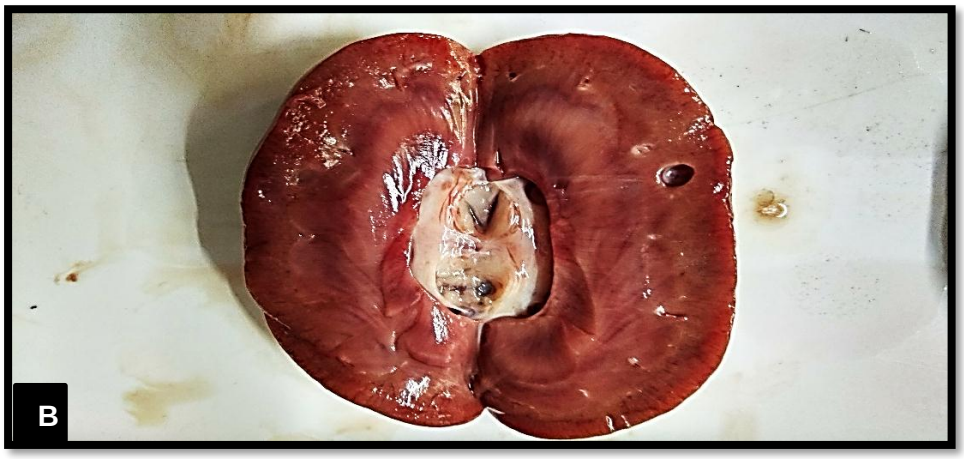
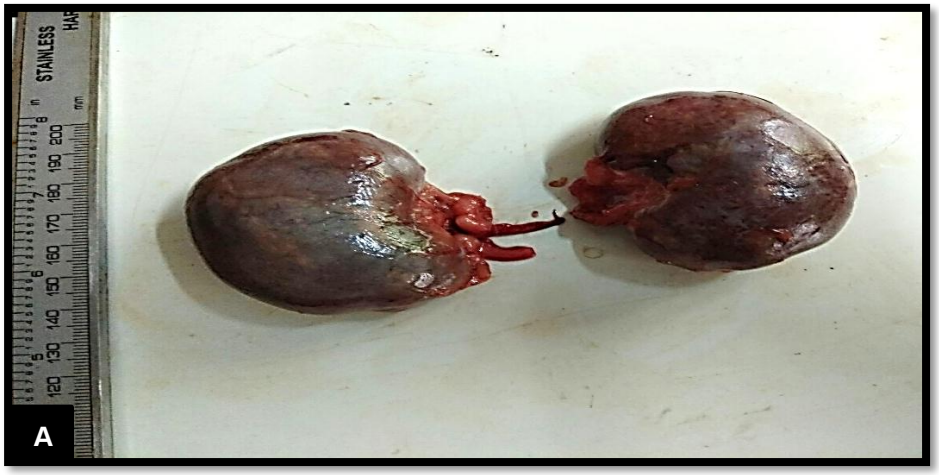


Plate 4(A): Congestion in cortical surface of kidney

Plate 4(B): cystic area in cortex of kidney



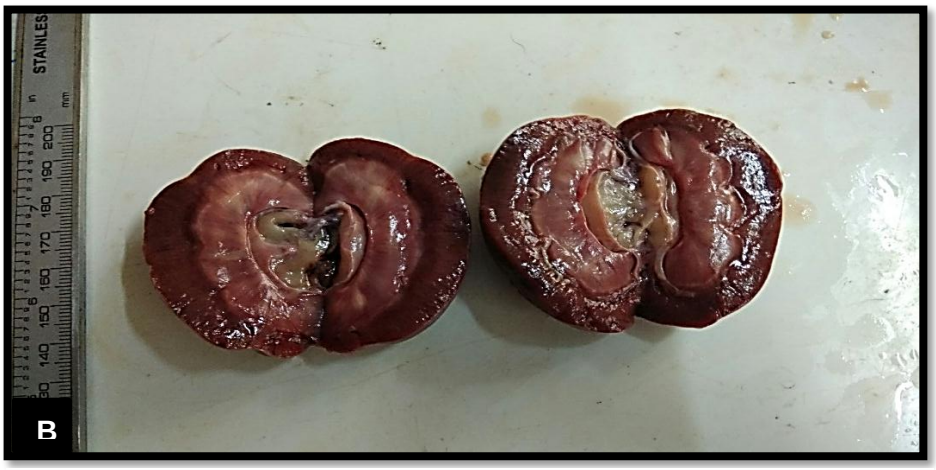


Plate 5(A): Capsule intact with kidney surface, contracted kidneys

Plate 5(B): Small granular contracted kidneys

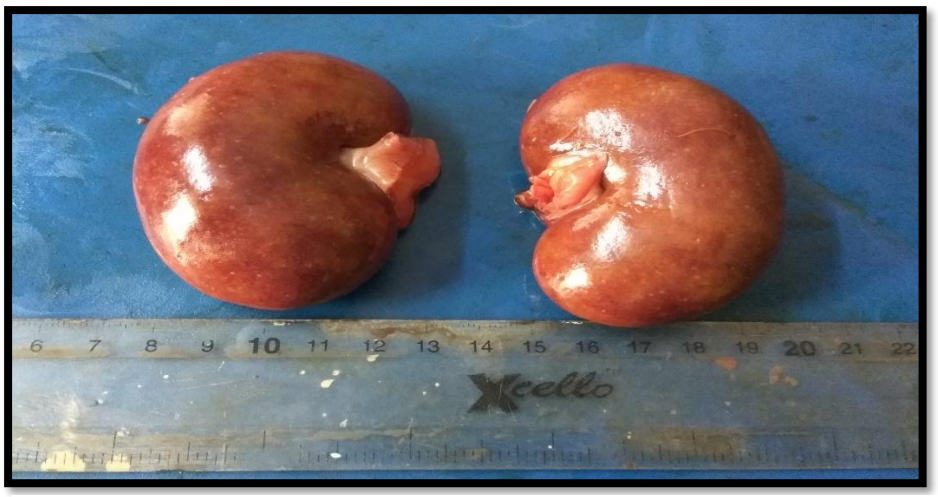


Plate 6: Pale foci on cortical surface of kidney

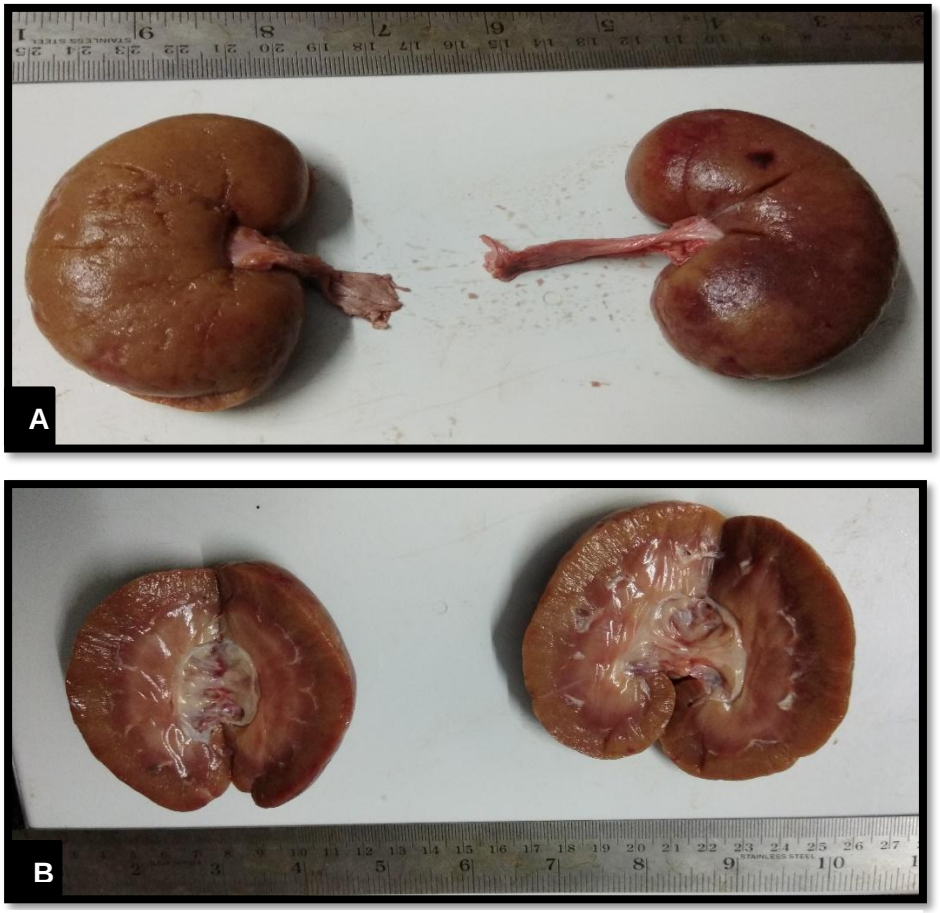


Plate 7(A): Haemorrhagic spots on cortical area

Plate 7(B): Congestion in cortical area

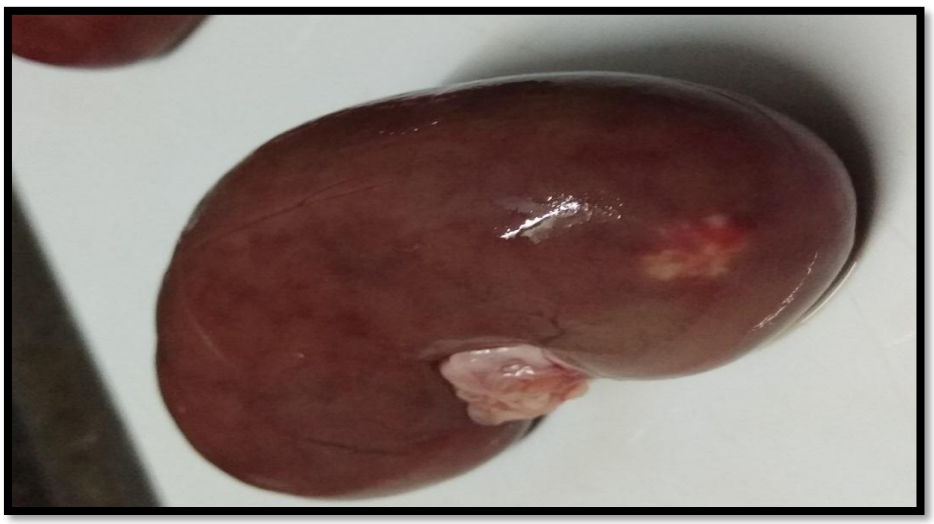


Plate 8: Necrotic patch on cortical area

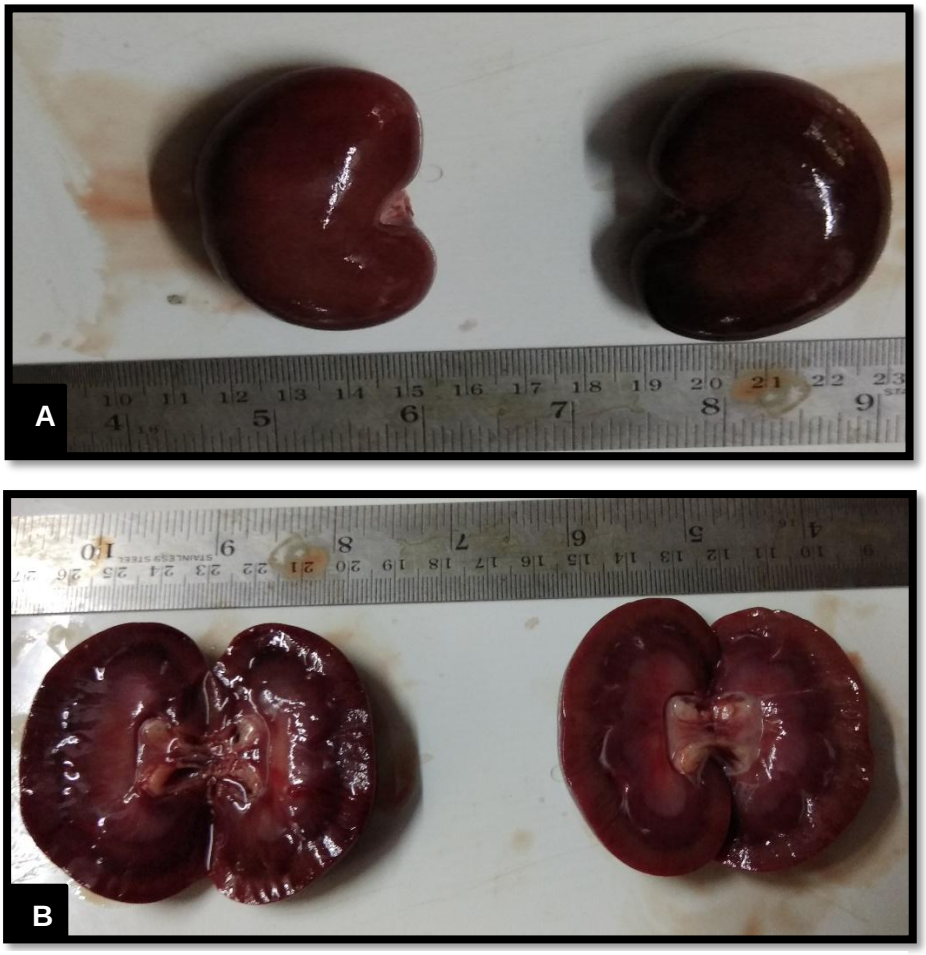


Plate 9(A): Congested kidneys

Plate 9(B): Congestion in corticomedullary area

Plate 10: Depressions on the cortical surface of the kidney

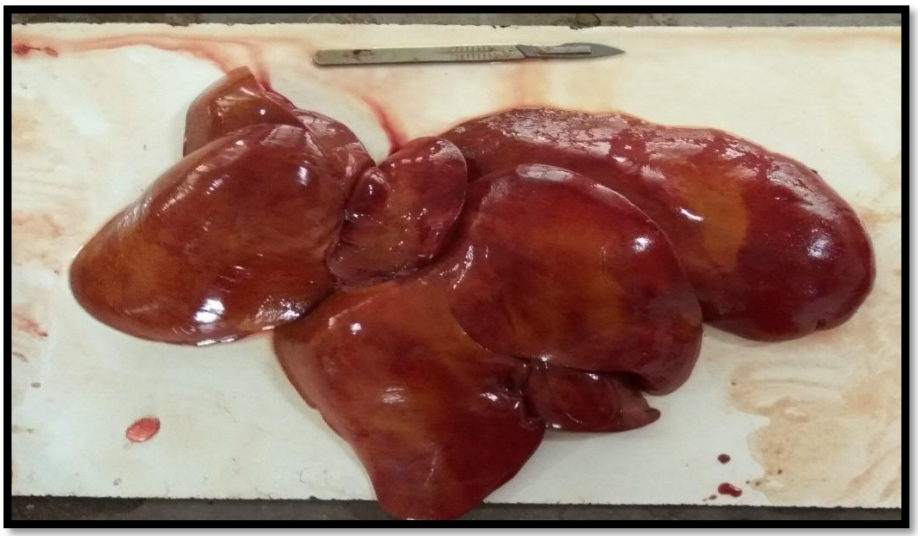
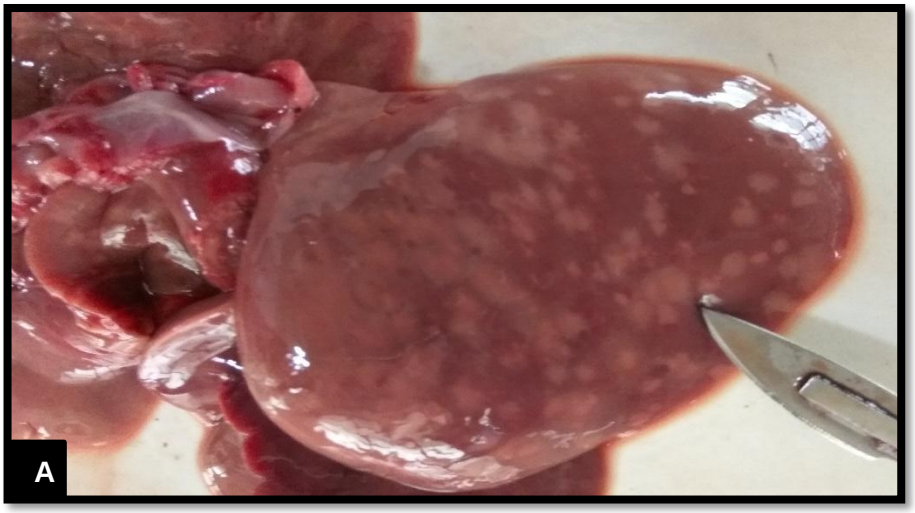


Plate 11: Congested, enlarged with rounding of borders and multifocal white patches were found on surface of liver



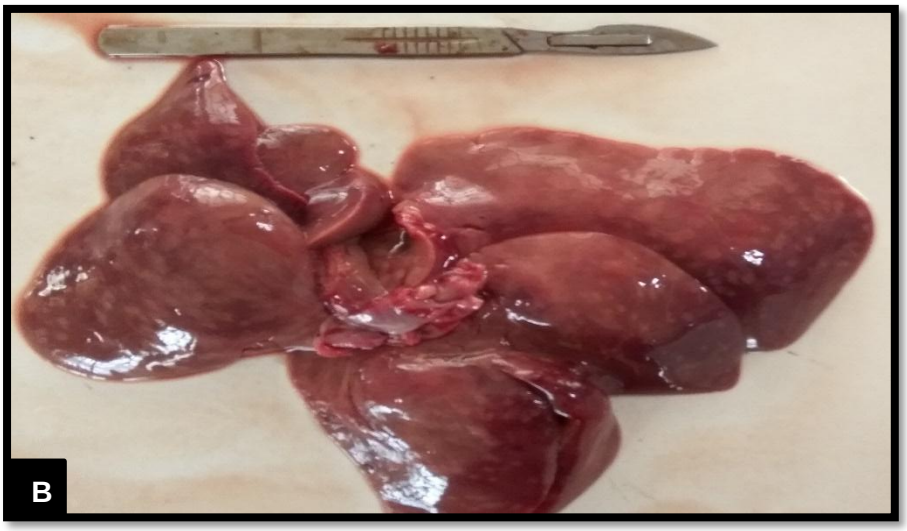


Plate 12(A & B): Diffuse necrotic foci on the surface of liver

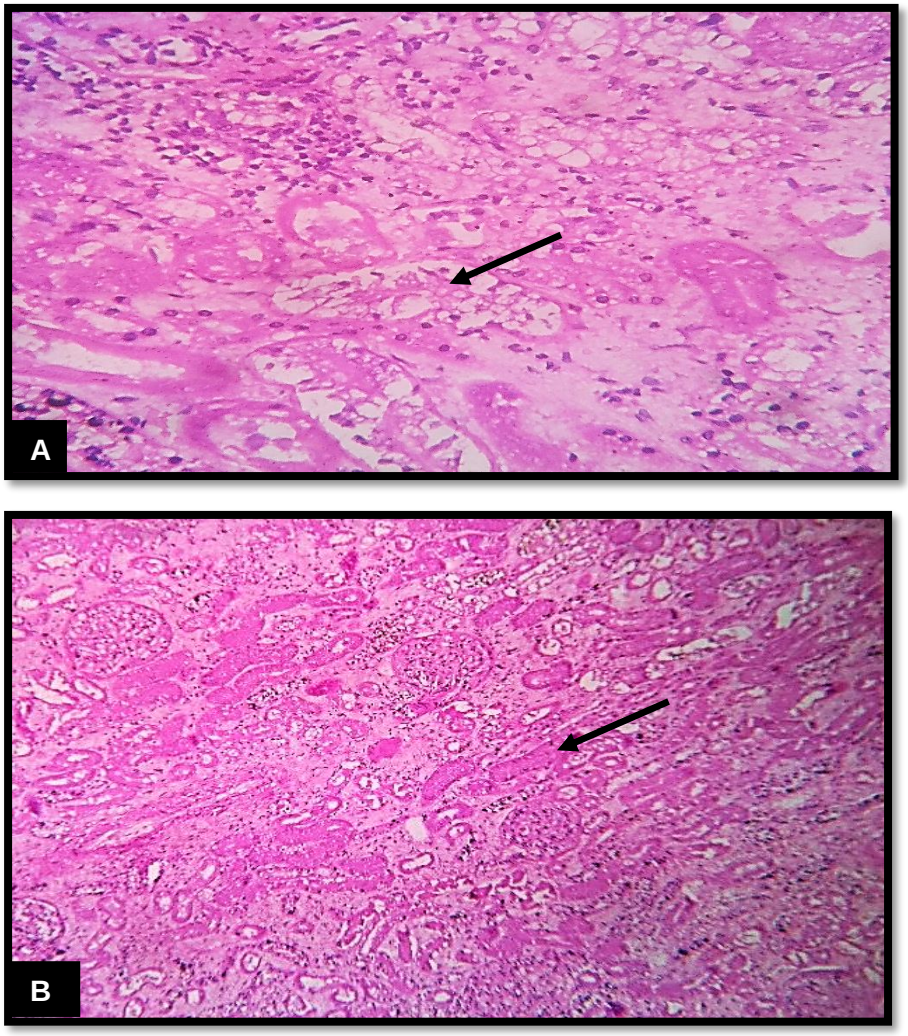


Plate 13(A): Tubular degeneration (H & E; 40 X)

Plate 13(B): Tubular dilatation and necrosis (H & E; 10 X)

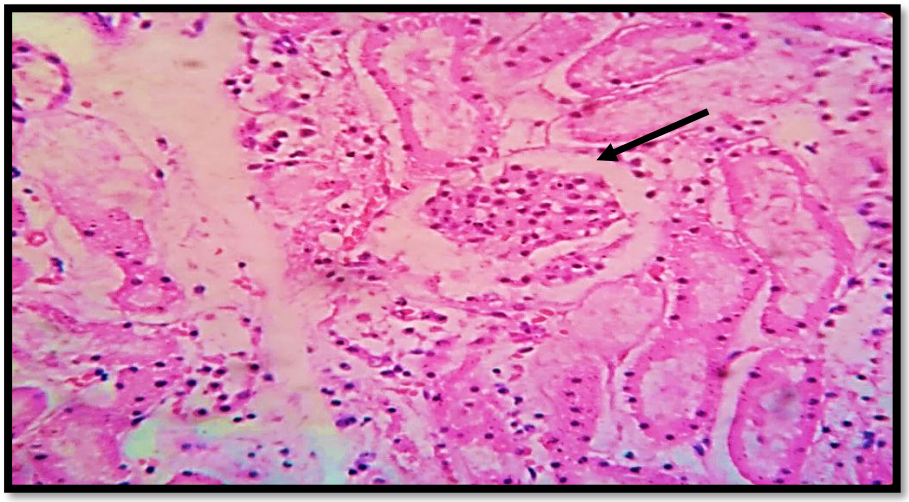


Plate 14: Atrophied glomerulus and degeneration of tubules (H & E; 40 X)

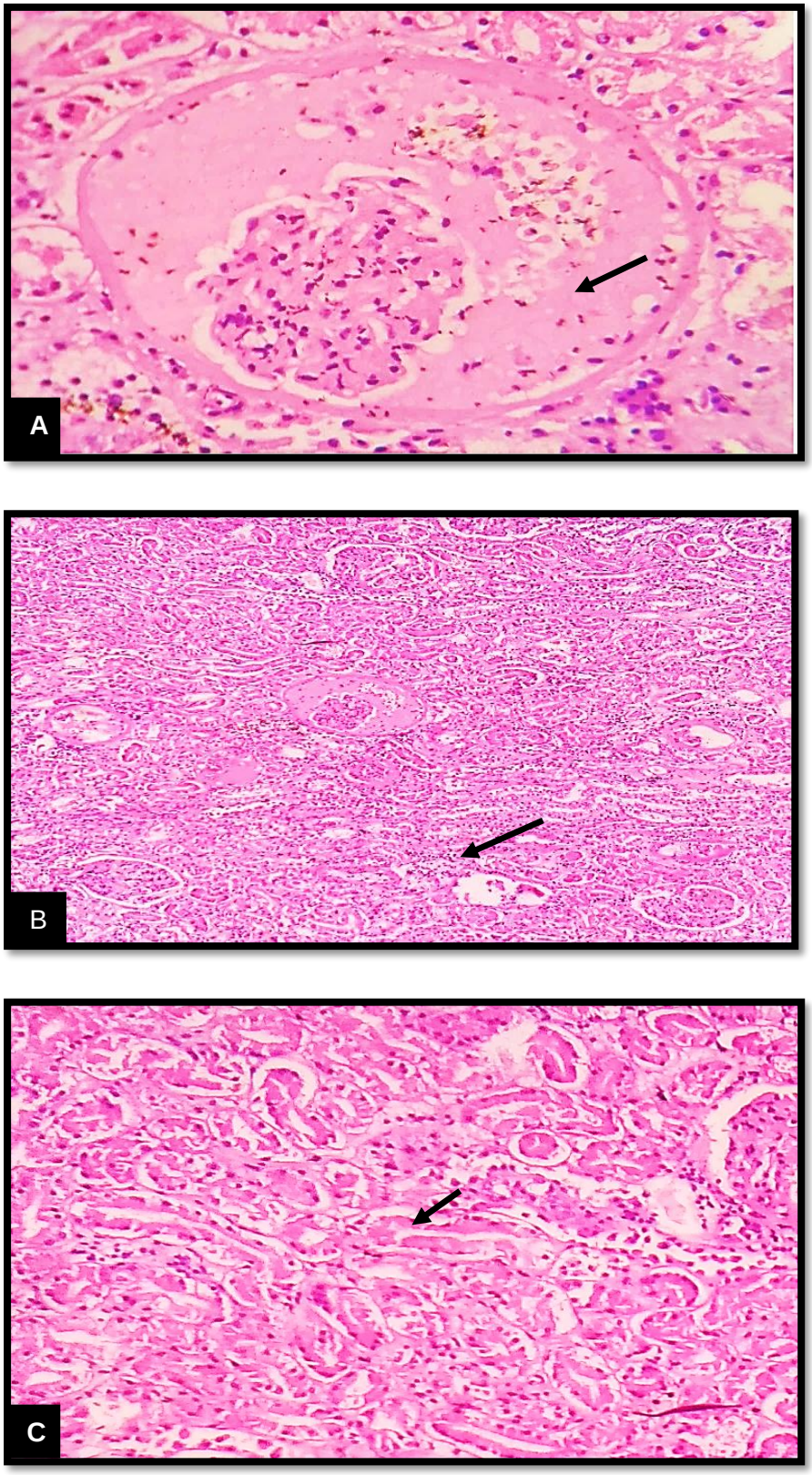
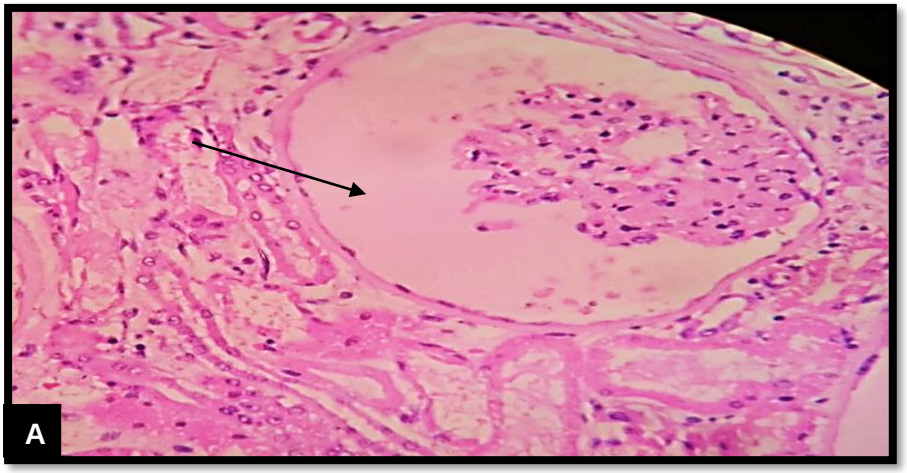
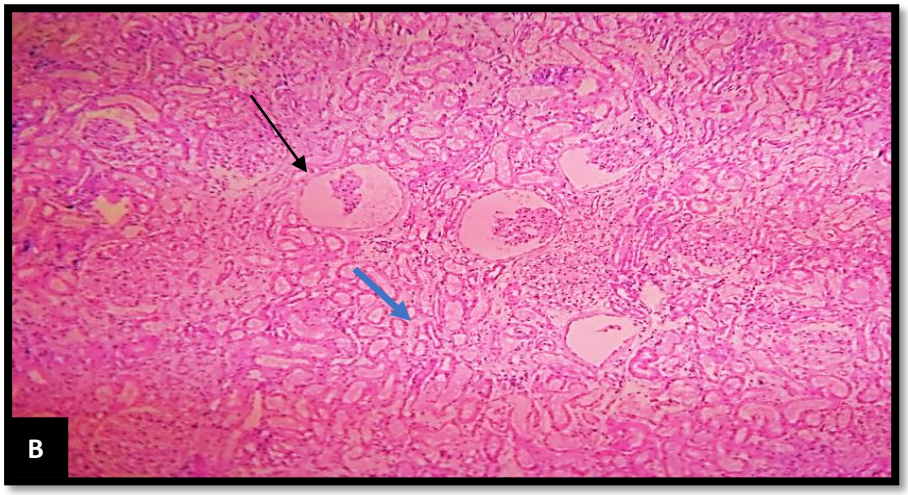


Plate 15(A): Pinkish fibrinous deposition in glomerulus (H & E; 40 X)

Plate 15(B): Infiltration of MNC in interstitial tissue (H & E; 10 X)

Plate 15(C): Tubular degeneration (H & E; 40 X)





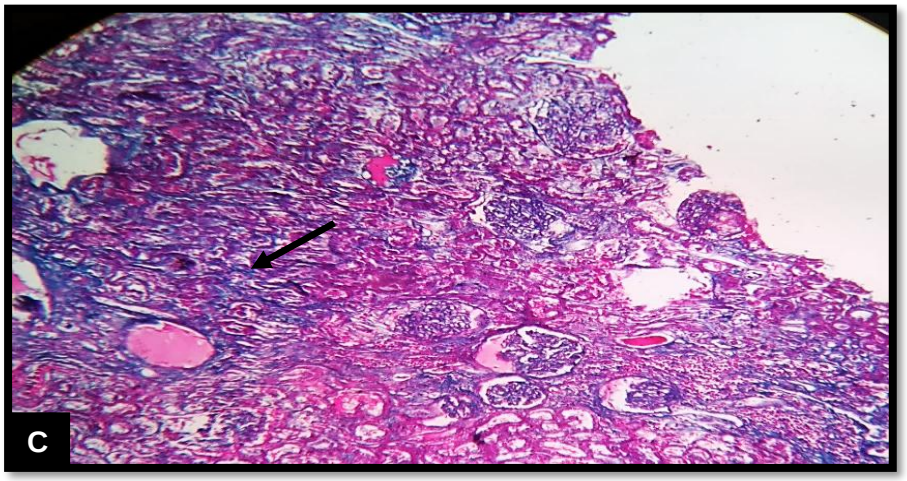


Plate 16(A): Atrophied glomerulus (H & E; 40 X)

Plate 16(B): Diffuse glomerular atrophy (black arrow) and degeneration of tubules (blue arrow) (H & E; 40 X)

Plate 16(C): Fibrous connective tissue in interstitial tissue (Masson's Trichrome stain; 40 X)

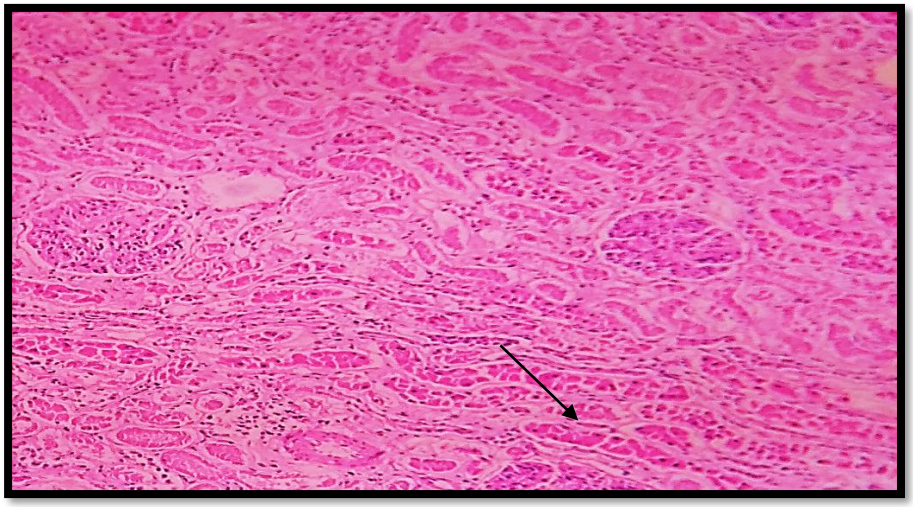


Plate 17: Tubular degeneration and coagulative necrosis (H & E; 10X)

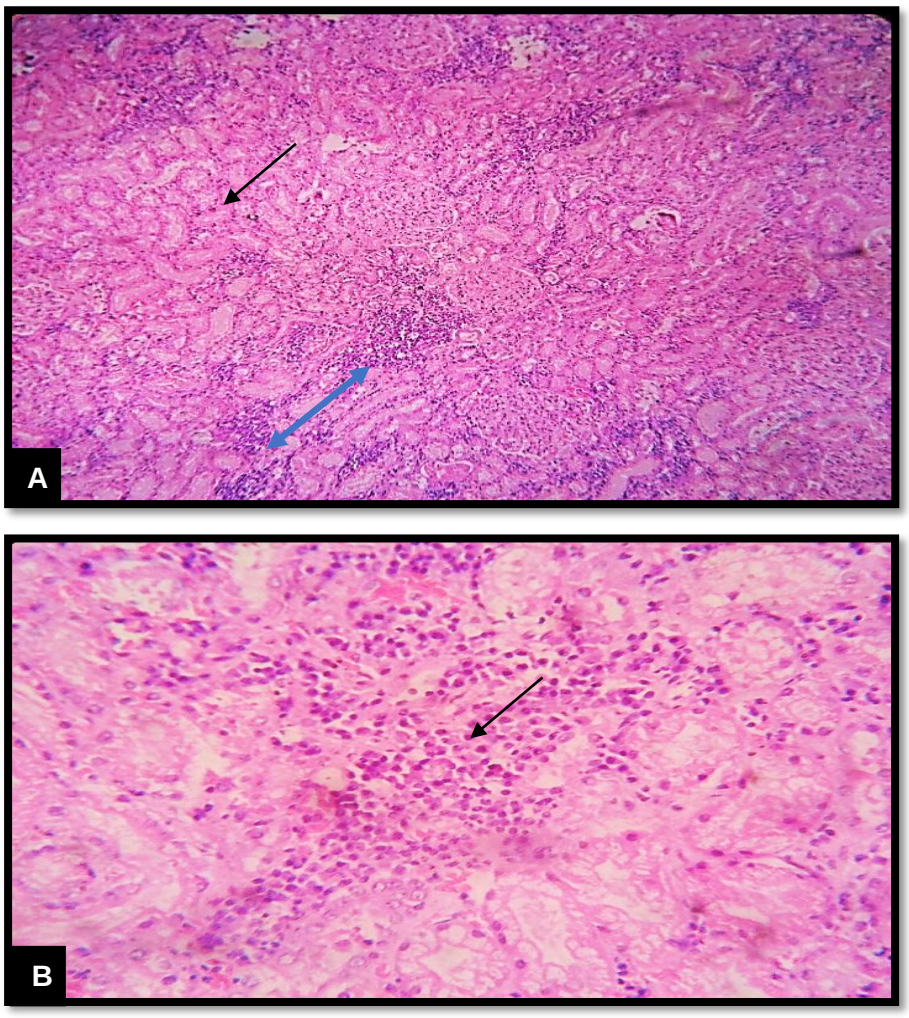


Plate 18(A): Multifocal areas of tubular degeneration (black arrow) and infiltration of MNC in interstitial tissue (double head blue arrow) (H & E; 10 X)

Plate 18(B): Infiltration of MNC (Plasma cells) (H & E; 40 X)

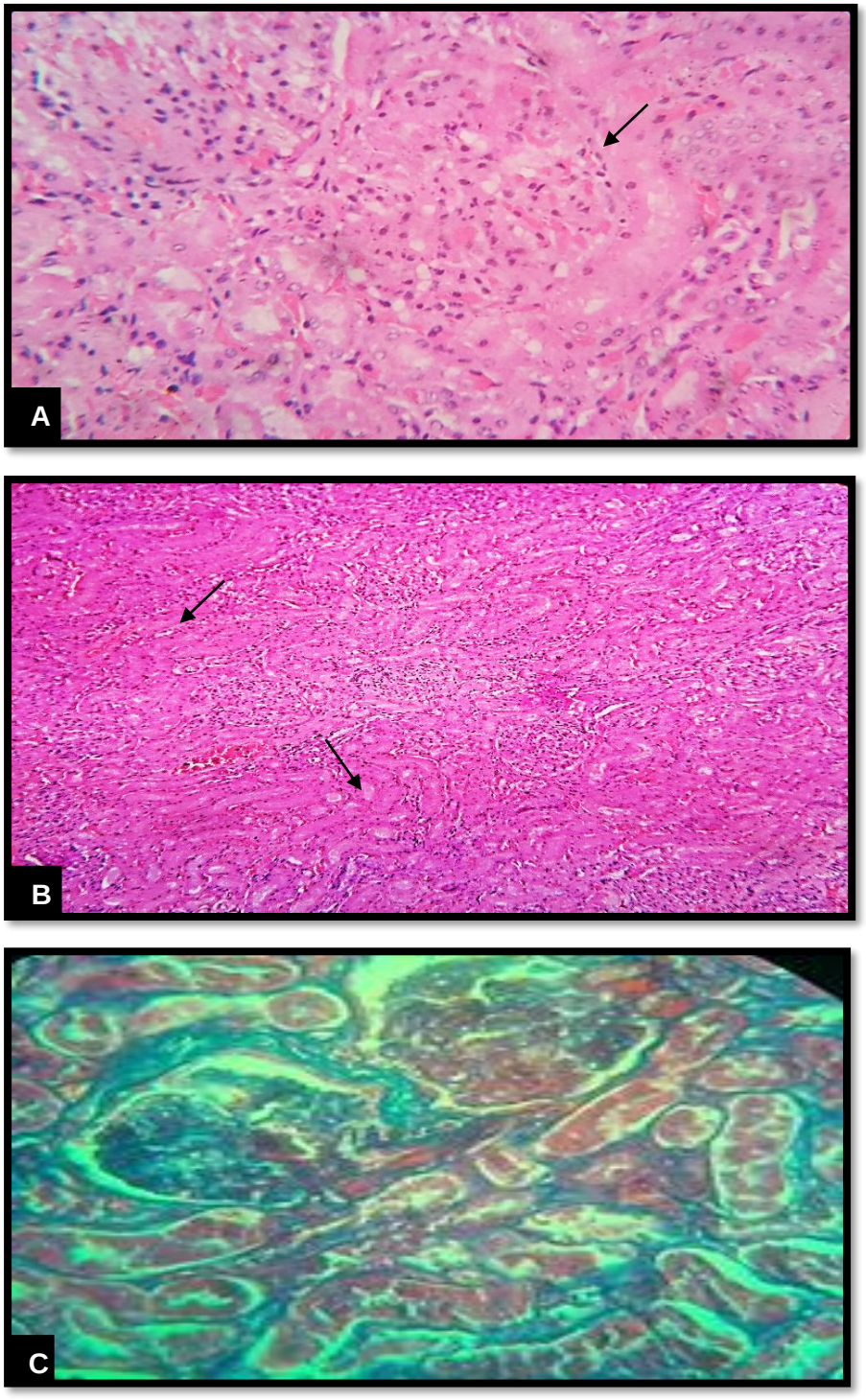


Plate 19(A): Adhesion of glomerulus with Bowman's capsule (H & E; 40 X)

Plate 19(B): Diffuse tubular cellular swelling and tubular coagulative necrosis (H & E; 10 X)

Plate 19(C): Fibrous tissue proliferation in glomerulus and interstitial tissue (Masson's Trichrome stain; 40 X)

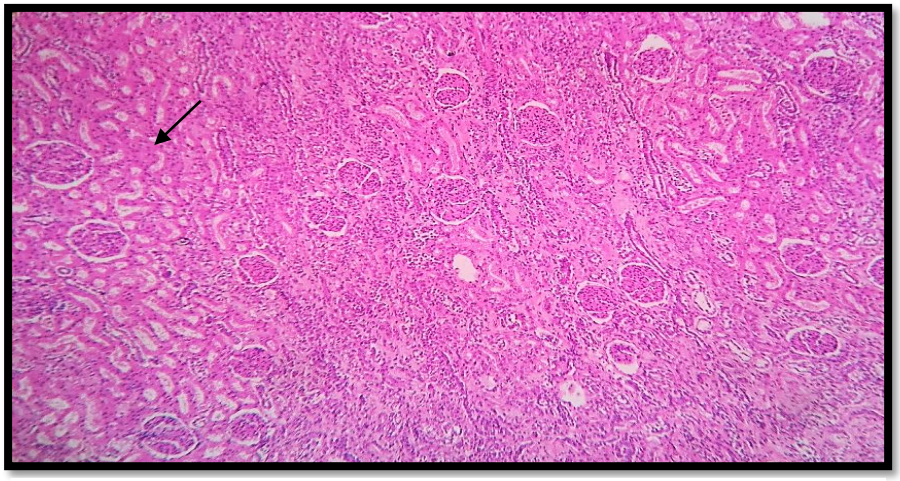


Plate 20: Multifocal tubular degeneration (H & E; 10 X)

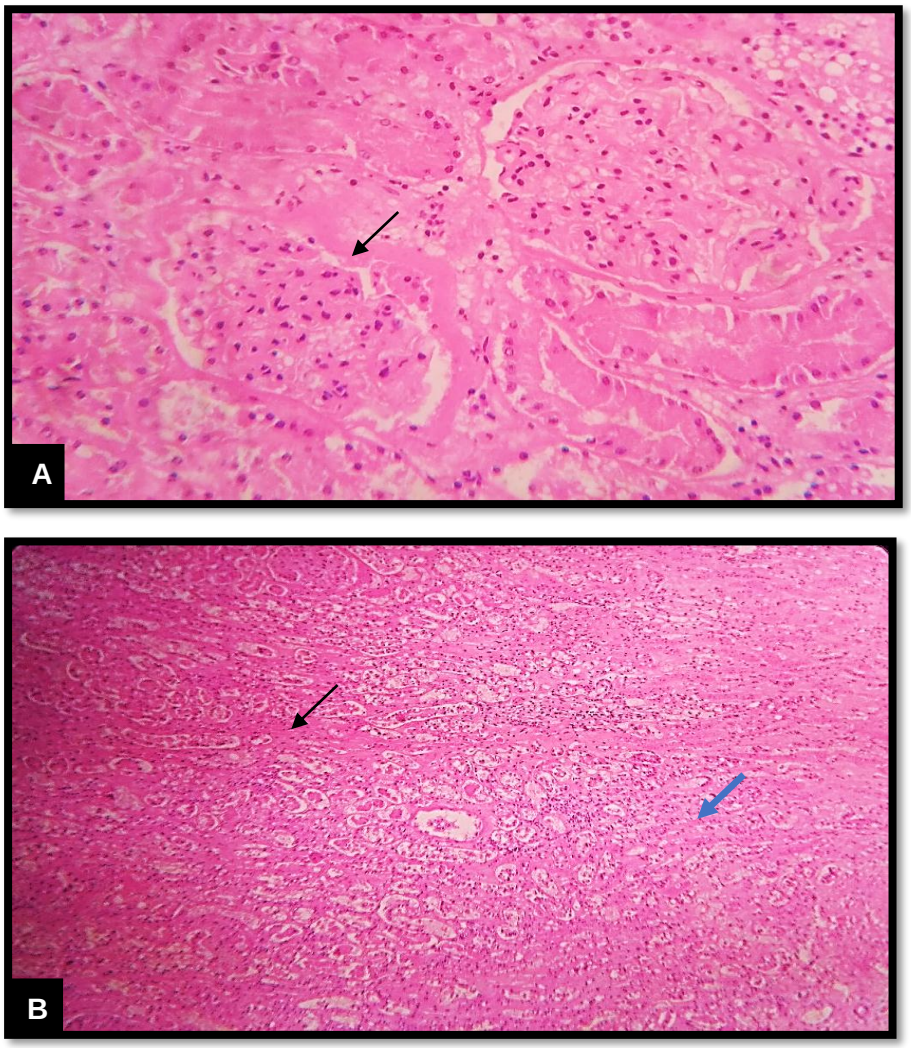


Plate 21(A): Thickening of Bowman's capsule (H & E; 40 X)

Plate 21(B): Tubular degeneration (blue arrow) and necrosis (black arrow) (H & E; 10 X)

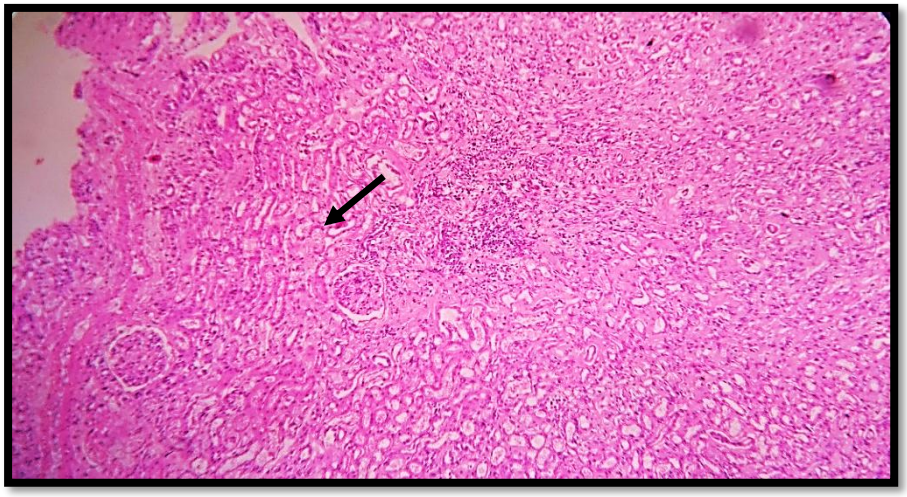


Plate 22: Tubular degeneration and fibrosis (H & E; 10 X)

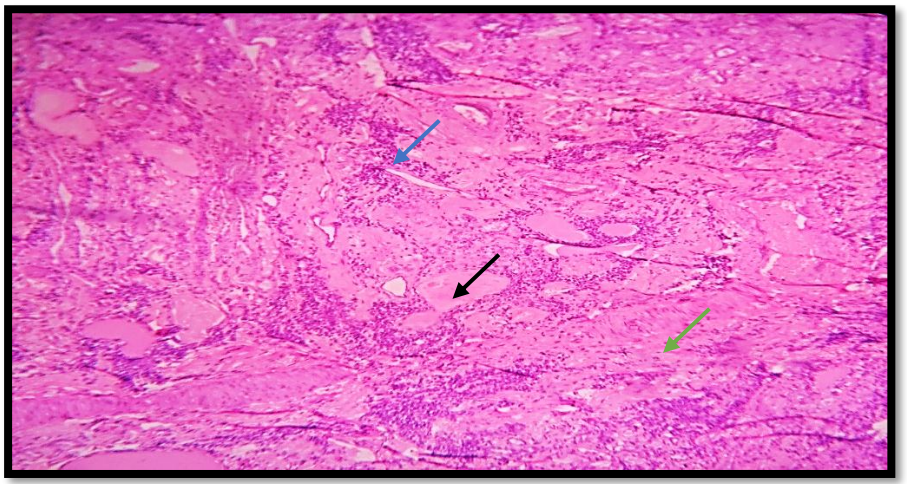


Plate 23: Multifocal cystic dilatation tubules (black arrow), infiltration of MNC (blue arrow) and fibrosis (green arrow) (H & E; 10 X)

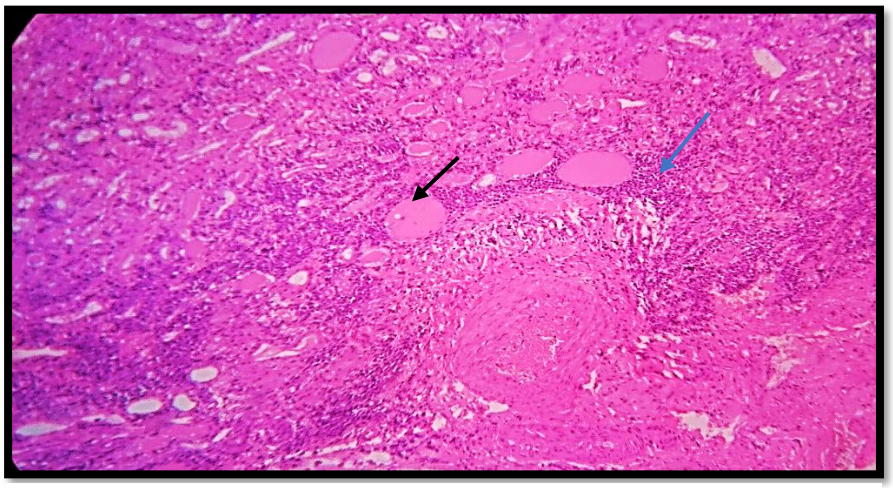


Plate 24: Diffuse cystic dilatation of tubules (black arrow), Multifocal infiltration of MNC (blue arrow) (H & E; 10 X)

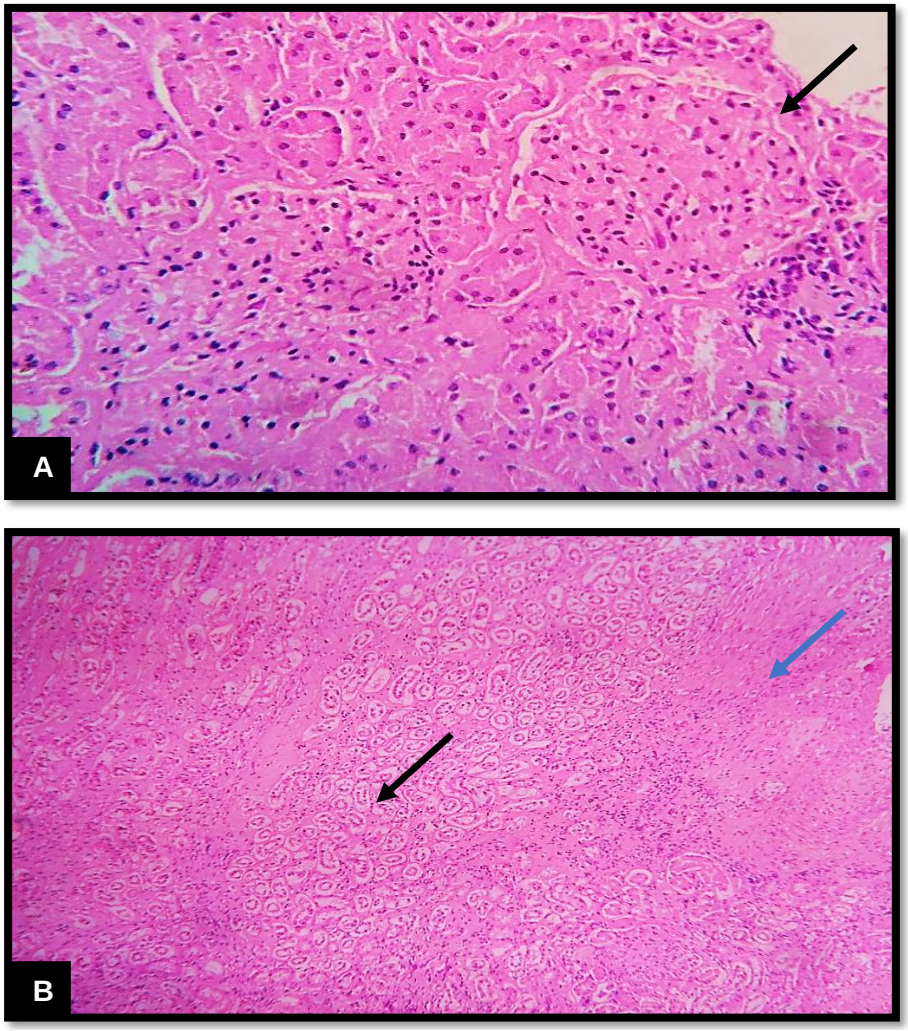


Plate 25(A): Hyalinization of glomerulus (H & E; 40 X)

Plate 25(B): Tubular necrosis (black arrow) and fibrous connective tissue proliferation in interstitial tissue (blue arrow) (H & E; 10 X)

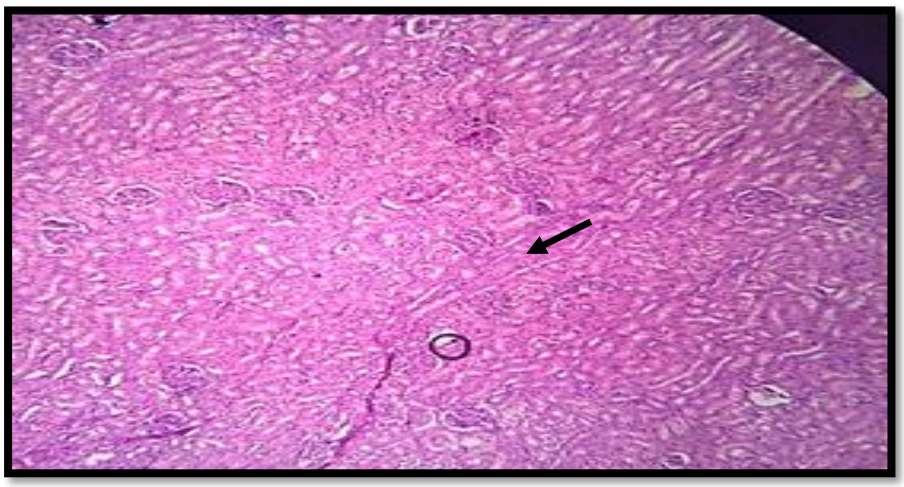


Plate 26: Multifocal tubular degeneration (H & E; 10 X)

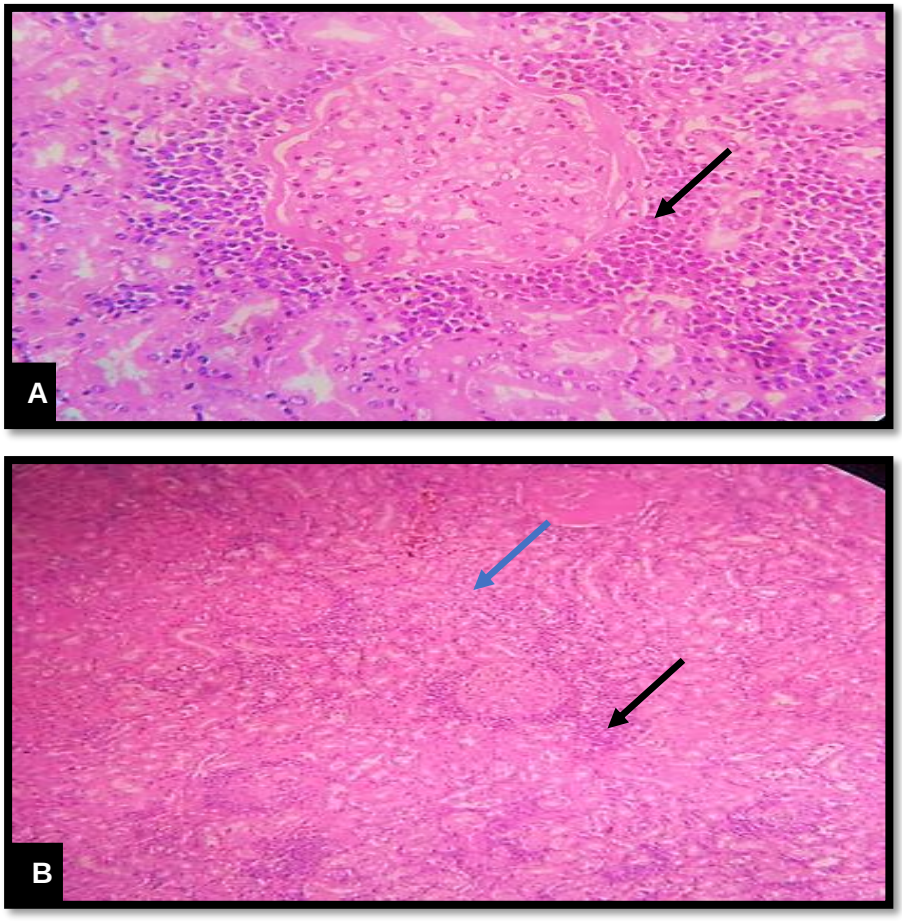


Plate 27(A): Infiltration of MNC (Plasma cells) (H & E; 40 X)

Plate 27(B): Diffuse tubular degeneration (blue arrow) and severe infiltration of MNC (black arrow) (H & E; 40 X)

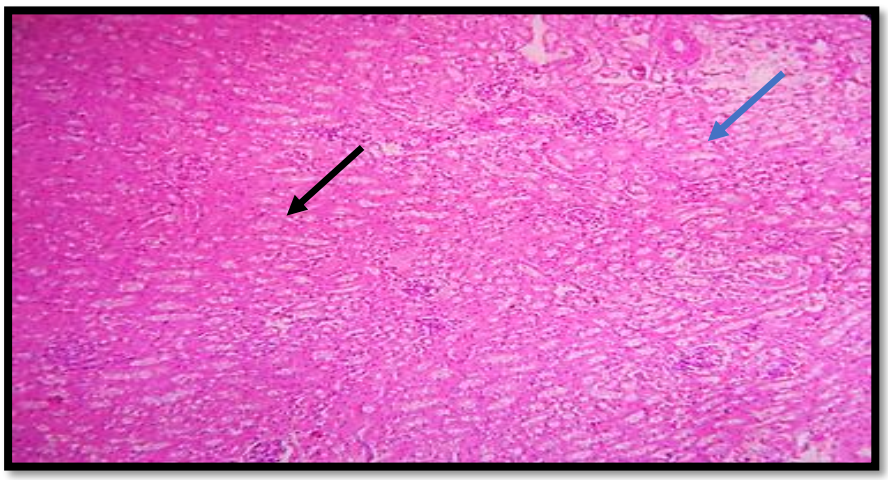
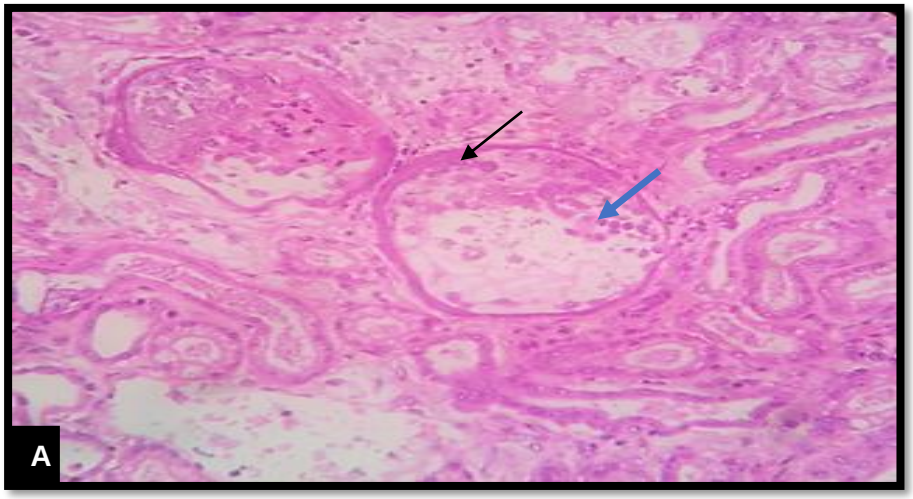
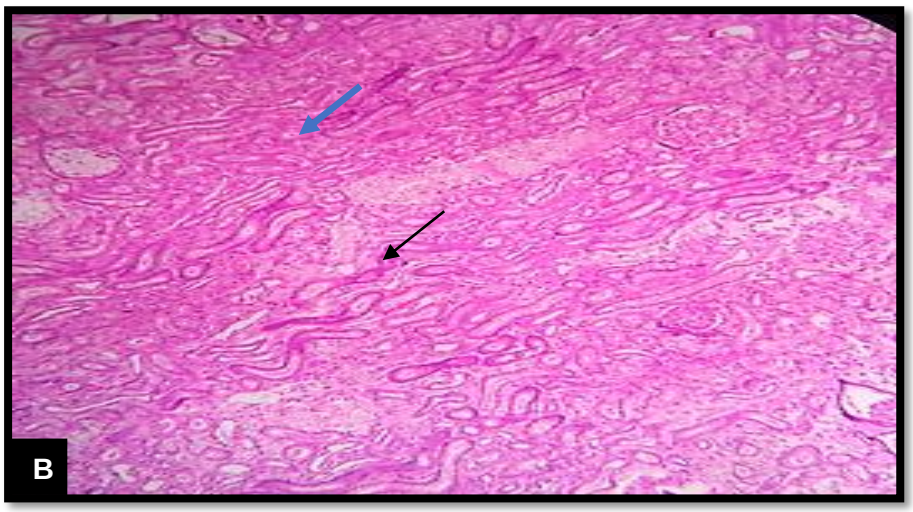


Plate 28: Multifocal areas of tubular coagulative necrosis (black arrow) and degeneration (blue arrow) (H & E; 40 X)





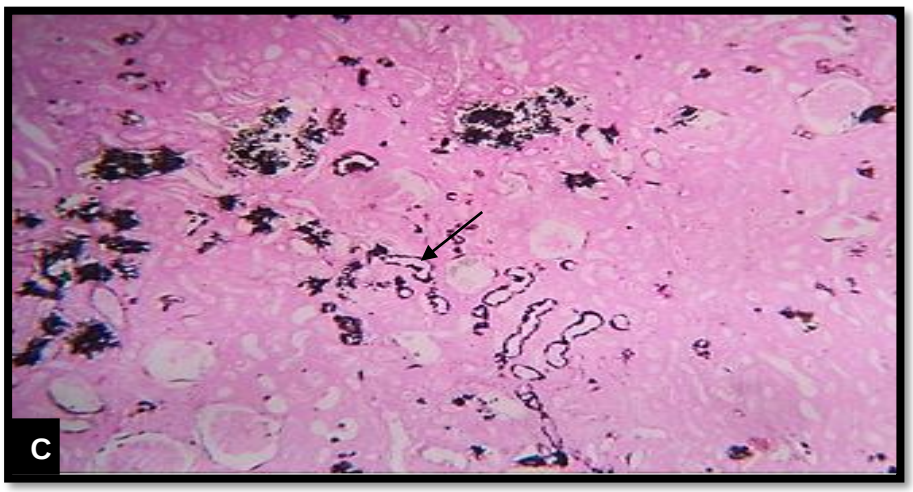


Plate 29(A): Multifocal glomerular atrophy (blue arrow) and calcification at Bowman's capsule (black arrow) (H & E; 40 X)

Plate 29(B): Typical tubular calcification (black arrow) and degeneration (blue arrow) (H & E; 40 X)

Plate 29(C): Calcification shown in black colour (Von kossa stain; 10 X)

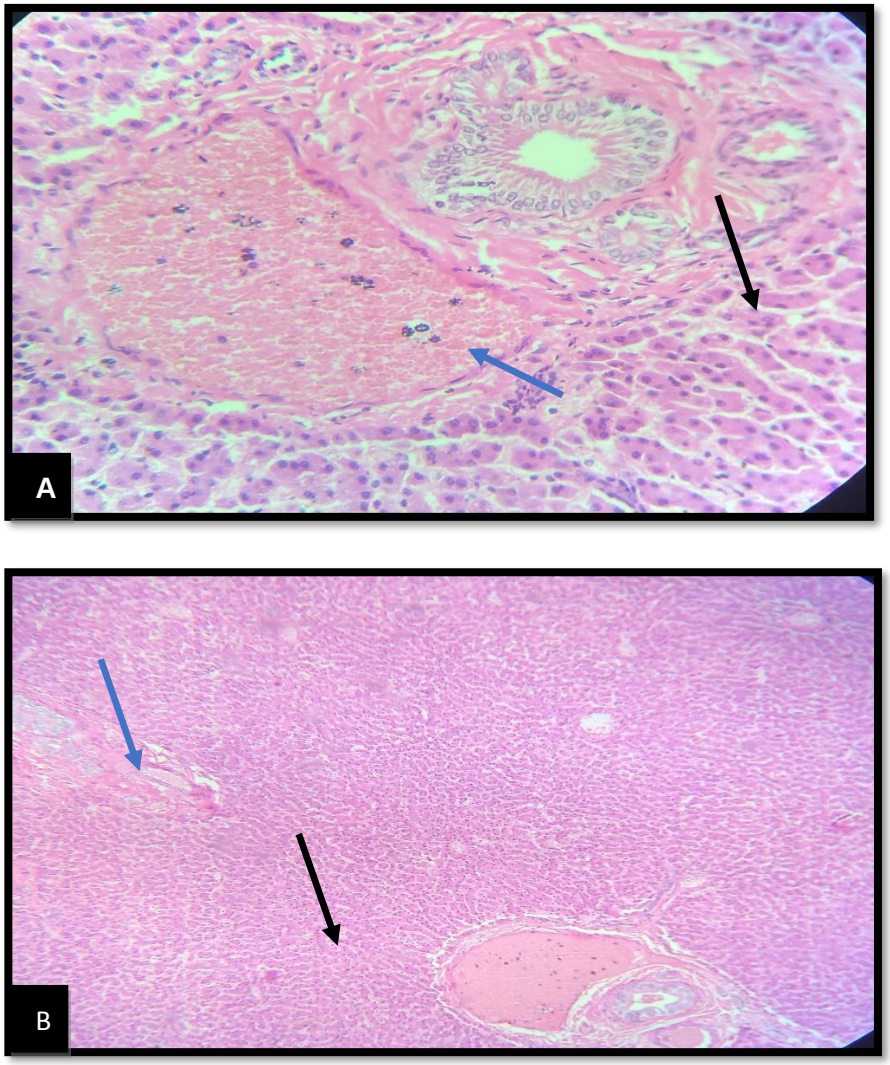


Plate 30(A & B): Degeneration (black arrow) and congestion (blue arrow)

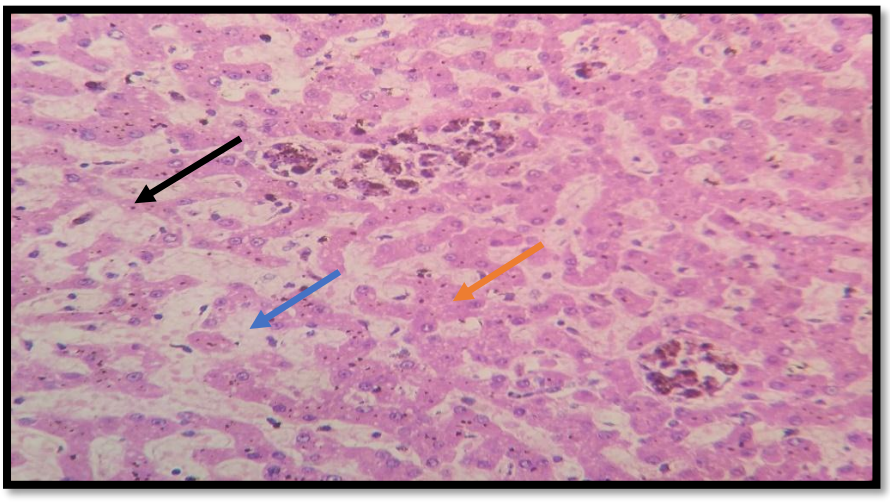


Plate 31: Atrophy of chords (black arrow), degeneration (orange arrow) and sinusoidal dilatation (blue arrow)

5. SUMMARY AND CONCLUSIONS

The present study, entitled ‘clinicopathological studies of canine renal dysfunction with special reference to lipocalin (NGAL) and beta 2 microglobulin (B2M) biomarkers’ was carried out in the Department of Veterinary Pathology, Bombay Veterinary College, Mumbai.

Sample survey of serum biochemistry data of the Department of Veterinary Pathology was carried out. Serum samples from the year 2015 - 2017 were analysed and those samples having serum creatinine value > 1.5 mg/dl were included in this study and age, sex and breed wise incidence were recorded.

Sample survey revealed that the highest incidence of renal disorders was recordedin the age group of 6-10 years (66.67%), followed by 0-5 years of age (22.22%) and11-15 years (11.11%). In the survey study, out of 27 cases, 18 cases were recorded in males (66.67%) and 9 cases were recorded in females (33.33%) and maximum incidence was observed in Labradors (29.62%) and non-descript breeds (25.92%) followed by Pomeranian (11.11%) and German shepherd (3.70%).

Fifty clinical cases of renal disorders were collected from affiliated medical ward, BSDPAH, Parel, and private clinics in and around Mumbai on the basis of serum creatinine. Three post mortem cases of dogs suspected for renal disorders were selected for this study. Detailed postmortem examination was carried out.

The 50clinical case were classified as ARF, CRF and Healthyand study revealed that the highest incidence recorded in the age group of 6-10 years (52.27%; ARF(73.33%) and CRF(26.67%)), followed by 0-5 years of age (34.1%; ARF(56.52%) and CRF(43.48%)) and lowest in the age group of 11-15 years (ARF:13.63%) respectively. Out of 44 cases, 28 cases were recorded in males (63.63%; ARF(64.29%) and CRF(35.71%)) and 16 cases were recorded in females (36.37%; ARF(75%) and CRF(25%)) whereas, maximum incidence was observed in Labradors (40.90%) and Pomeranian breeds (15.90%) followed by non-descript (13.63%), and German shepherd (9.09%).

There was significant decrease in the values of Hb, PCV, TECmainly in CRF cases with 40% of cases showing leukocytosis. Serum creatinine value was increased in dogs which are affected with renal dysfunction. There was no significant difference recorded in hematological and biochemical parameters in between different age and breeds.

Fifty samples were processed for lipocalin (NGAL) by ELISA (BIOPORTO).There was statistically significant difference in the values of NGAL within the groups ($P \leq 0.05$). Dogs with AKI had the highest serum NGAL concentration when compared with CKD and healthy groups, whereas dogs with CKD had higher concentration of serum NGAL than that of healthy group.

Amongst these 50 samples, 14 CRF samples were processed for B2M by ELFA (mini VIDAS) for presence of amyloidosis and glomerular damage. The result for B2M was negative as all the 14 values were in normal range (0.3 to 3.0 mg/l).

Nephritis was classified histologically according to Ganti and Rao (1968). Three kidney samples of clinical cases and 25 kidney samples of departmental necropsy cases were processed for histopathology. Out of three, 1 case was diagnosed as membranous glomerulonephritis, 2 cases as interstitial nephritis. Among departmental necropsy cases 15 cases were diagnosed as interstitial nephritis (60%), 2 cases were diagnosed as glomerulonephritis (8%), in 1 case (4%) calcification observed and in remaining cases degenerative and necrotic changes has been observed.

Based on results obtained in the present study, following conclusions can be drawn.

1) It was found that renal disorders were seen in all age groups but dogs of age 6 - 10 years were mostly affected. Males were more prone to renal disorders than females and incidence of renal disorders was more in Labradors followed by non-descript.
2) There was reduction in the values of Hb, PCV, TEC mainly in CRF cases along with leukocytosis. Serum creatinine and BUN value was increased in dogs which are affected with renal dysfunction.

3) It was found that serum NGAL was higher in ARF cases followed by CRF and Healthy. B2M was in normal range as there was no amyloidosis.
4) Macroscopically, in most of the cases, kidneys were smaller in size, pale to grey in color, were hard to cut and capsule was very hard to peeled off. The cortex was shrunken. The surface was uneven due to irregular contraction of the fibrous tissue.
5) Out of 3 clinical necropsy cases of canine renal disorders, 1 case was of membranous glomerulonephritis and 2 cases were of interstitial nephritis.
6) Among 25 departmental necropsy cases 15 cases were diagnosed as interstitial nephritis (60%), 2 cases were diagnosed as glomerulonephritis (8%), in 1 case (4%) calcification observed and in remaining cases degenerative and necrotic changes were observed.

BIBLIOGRAPHY

Adiyanti, S. S. and T. Loho (2012) Acute kidney injury (AKI) biomarker. *Acta Med Indones*, ***44*(3):** 246-55.

Adin, C. A. and L. D. Cowgill, (2000) Treatment and outcome of dogs with leptospirosis: 36 cases (1990–1998). *Journal of the American Veterinary Medical Association*, ***216*(3),** 371-375.

Ahn, H. J. and C. Hyun (2013) Evaluation of serum neutrophil gelatinase-associated lipocalin (NGAL) activity in dogs with chronic kidney disease. *Veterinary Record*, vetrec-2013.

Ahuja, M., A. Basu, G. Gyamlani and M. Moxey (2010) 17: Serum Beta 2 Microglobulin Levels in Patients on Chronic Hemodialysis. *American Journal of Kidney Diseases*, *55*(4), B36.

Bartlett, P. C., J. W. Van Buren, A. D. Bartlett and C. Zhou (2010) Case-control study of risk factors associated with feline and canine chronic kidney disease. Veterinary medicine international, 2010.

Bellomo, R., C. Ronco, J. A. Kellum, R. L. Mehta and P. Palevsky (2004) Acute renal failure–definition, outcome measures, animal models, fluid therapy and information technology needs: The Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Critical care*, ***8*(4)**, R204.

Benjamin. M. M., (1985) Outline of Veterinary Clinical Pathology, 3rd edn. Kalyani Publishers, New Delhi- Ludhiana: 180-212.

Bernier, G. M. (1980). β2-Microglobulin: Structure, Function and Significance. *Voxsanguinis*, ***38*(6),** 323-327.

Bjorling, D. E. (2003) The urethra. Textbook of small animal surgery, 1638-1651.

Bolignano, D., V. Donato, G. Coppolino, S. Campo, A. Buemi, A. Lacquaniti, and M. Buemi (2008) Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a Marker of Kidney Damage. *American Journal of Kidney Diseases*, ***52*(3)**: 595–605. https://doi.org/10.1053/j.ajkd.2008.01.020

Booth, K. (1990) A case of juvenile nephropathy in a Newfoundland dog. *Veterinary Record*, *127*(24): 596-597.

Bradea, A., M. Codreanu, C. Vlagioiu and V. Simion (2013) Hematologic aspects in chronic kidney disease (CDK) in dogs. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca,Veterinary Medicine, **70(2).**

Brown, S. A., J. A. Barsanti and W. A. Crowell (1985) Gentamicin-associated acute renal failure in the dog. Journal of the American Veterinary Medical Association, **186(7)**: 686.

Chandrasekaran, D., S. Prathaban, P. Dhanabalan, Balachandran, C. Balachandran, M. B. Murali & K. S. Venkataraman (2011). Pathological Changes in Canine Leptospirosis. *Tamil Nadu J Veterinary & Animal Sciences*, ***7***, 180-183.

Chew, D. J., S. P. DiBartola, J. T. Boyce, J. H. Hayes and J. J. Brace (1983) Juvenile renal disease in Doberman Pinscher dogs. Journal of the American Veterinary Medical Association, **182(5)**: 481-485.

Cobrin, A. (2013). *Measurement of Serum and Urine Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Dogs with Chronic Kidney Disease, Lymphosarcoma, Carcinoma and Induced Endotoxemia to Assess Diagnostic Utility of NGAL in Dogs with Chronic Kidney Disease* (Doctoral dissertation).

Cook, S. M., D. F. Dean, D. L. Golden, J. E. Wilkinson and T. L. Means (1993) Renal failure attributable to atrophic glomerulopathy in four related rottweilers. Journal of the American Veterinary Medical Association, **202(1)**: 107.

Cortellini, S., L. Pelligand, H. Syme, Y. M. Chang and S. Adamantos (2015). Neutrophil Gelatinase-Associated Lipocalin in Dogs with Sepsis Undergoing Emergency Laparotomy: A Prospective Case–Control Study. *Journal of veterinary internal medicine*, ***29*****(6),** 1595-1602.

Cowgill, L. D. (1992) Pathophysiology and management of anemia in chronic progressive renal failure. *Semin Vet Med Surg (Small Anim)*, ***7*****(3)**, 175–182.

Cowland, J. B. and N. Borregaard (1997) Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. *Genomics*, ***45*****(1)**: 17-23.

Cruz, D. N., M. de Cal, F. Garzotto, M. A. Perazella, P. Lentini, V. Corradi and C. Ronco, (2010). Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. *Intensive care medicine*, ***36*****(3),** 444-451.

Daure, E., M.C. Belanger, G. Beauchamp and C. Lapointe (2013) Elevation of neutrophil gelatinase-associated lipocalin (NGAL) in non-azotemic dogs with urinary tract infection. *Research in veterinary science*, ***95*****(3):** 1181-1185.

DiBartola, S. P., (2005) "Renal disease: clinical approach and laboratory evaluation." *Textbook of veterinary internal medicine*2 (2005).

De Geus, H. R. H., J. Bakker, E. M. E. H. Lesaffre and J. L. M. L. Le Noble (2011) Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients. *American Journal of Respiratory and Critical Care Medicine*, ***183*****(7)**: 907–914.

Dent, C. L., Q. Ma, S. Dastrala, M. Bennett, M. M. Mitsnefes, J. Barasch and P. Devarajan (2007) Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. *Critical Care (London, England)*, ***11*****(6)**: R127.

Devarajan, P. (2008) Neutrophil gelatinase-associated lipocalin (NGAL): A new marker of kidney disease. *Scandinavian journal of clinical and laboratory investigation*, ***68***(sup241): 89-94.

Doumas, B. T., R. L. Arend and P. C. Pinto (1972) Estimation of serum albumin, in Standard Methods of Clinical Chemistry. Academic Press Chicago, **7** : 175-189.

Eubig, P. A., M. S. Brady, S. M. Gwaltney-Brant, S. A. Khan, E. M. Mazzaferro, and C. M. Morrow (2005). Acute renal failure in dogs after the

ingestion of grapes or raisins: a retrospective evaluation of 43 dogs (1992–2002). *Journal of veterinary internal medicine*, ***19*****(5),** 663-674.

Eknoyan, G., N. Lameire and R. Barsoum (2004) "The burden of kidney disease: improving global outcomes". Kidney Int. **66**: 1310–4.

Fagundes, C., M. N. Pépin, M. Guevara, R. Barreto, G. Casals, E. Sola and E. Poch, (2012). Urinary neutrophil gelatinase-associated lipocalin as biomarker in the differential diagnosis of impairment of kidney function in cirrhosis. *Journal of hepatology*, ***57*****(2)**, 267-273.

Gansevoort, R. T., R. Correa-Rotter, B. R. Hemmelgarn, T. H. Jafar, H. J. L. Heerspink, J. F. Mann and C. P. Wen (2013) Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. The Lancet, **382(9889)**: 339-352

Ganti A. Sastry and P. Rama Rao (2006) Veterinary Pathology, seventh edition. CBS publishers and distributors, 383.

George, E., G. E. Lees., R. E. Cianciolo and F. J. Clubb (2011) Renal Biopsy and Pathologic Evaluation of Glomerular Disease. Topics in Companion Animal Medicine **26(3):**143-153.

Grauer, G. F. and I.F. Lane (1995) “Acute Renal Failure: Ischemic and Chemical Nephrosis” Canine and Feline Nephrology and Urology, Osborne C A and Finco D R (Eds.), pp. 441-459, Lea and Febiger Book, Williams and Wilkins, USA.

Greco, D. S. (2001) Congenital and inherited renal disease of small animals. Veterinary Clinics of North America: Small Animal Practice, **31(2)**: 393-399.

Hall, P. W. and M. Vasiljevic (1973) Beta2-microglobulin excretion as an index of renal tubular disorders with special reference to endemic Balkan nephropathy. *The Journal of laboratory and clinical medicine*, *81*(6), 897-904.

Hall, P. W., M. Chung-Park, C. V. Vacca, M. London and A. Q. Crowley (1982). The renal handling of beta2-microglobulin in the dog. *Kidney international*, ***22*****(2),** 156-161.

Harley, L. and C. Langston (2012) Proteinuria in dogs and cats. The Canadian Veterinary Journal, **53(6)**: 631.

Heiene, R., V. Kristiansen, J. Teige and J. H. Jansen (2007) Renal histomorphology in dogs with pyometra and control dogs, and long term clinical outcome with respect to signs of kidney disease. *Acta Veterinaria Scandinavica*, ***49*(1)**, 13.

Hsu, W. L., Y. S. Lin, Y. Y. Hu, M. L. Wong, F. Y. Lin and Y. J. Lee (2014[A]) Neutrophil Gelatinase–Associated Lipocalin in Dogs with Naturally Occurring Renal Diseases. Journal of veterinary internal medicine, **28(2)**: 437-442.(a)

Hsu, W. L., H.C. Chiou, K. C. Tung, G. Belot, A. Virilli, M.L. Wong, and Y. J. Lee (2014[B]) The different molecular forms of urine neutrophil gelatinase-associated lipocalin present in dogs with urinary diseases. *BMC veterinary research*, ***10*(1):** 202. (b)

Jeong, W. I., S. H. Do, D. H. Jeong, J. Y. Chung, H. J. Yang, D. W. Yuan and K. S. Jeong (2006) Canine renal failure syndrome in three dogs. Journal of veterinary science, **7(3)**: 299-301.

Kandula, S. and S. K. Karlapudi (2014) PREVALENCE OF RENAL DISORDERS IN DOGS—A CLINICAL STUDY. *Int. J. Agric.Sc & Vet.Med*., 2**(3).**

Kandula, S and S. K. Karlapudi (2015) HAEMATO-BIOCHEMICAL CHANGES IN DOGS WITH RENAL INSUFFICIENCY AND ITS DIAGNOSTIC SIGNIFICANCE. Animal Science, **9(4)**.

Karlsson, F. A., L. Wibell and P.E. Evrin (1980) beta 2-Microglobulin in clinical medicine. *Scandinavian journal of clinical and laboratory investigation. Supplementum*, ***154*, 27-37.**

Kjeldsen, L., A. H. Johnsen, H. Sengeløv and N. Borregaard (1993) Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. Journal of Biological Chemistry, **268(14)**: 10425-10432.

Kovarikova, S. (2015) Urinary biomarkers of renal function in dogs and cats: a review. *Veterinarni Medicina*, ***60*(11)**: 589-602.

Koyner, J. L., V. S. Vaidya, M. R. Bennett, Q. Ma, E. M. Worcester, S. A. Akhter and J. V. Bonventre (2010) Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. Clinical Journal of the American Society of Nephrology, CJN-00740110.

Kumar, V., A. K. Abbas, N. Fausto and J. C. Aster (2014) Robbins and Cotran pathologic basis of disease, professional edition e-book..Elsevier Health Sciences.

Laroute, V., V. Chetboul, L. Roche, C. Maurey, G. Costes, J. L. Pouchelon and H. P. Lefebvre (2005) Quantitative evaluation of renal function in healthy Beagle puppies and mature dogs. Research in veterinary science,**79(2)**: 161-167.

Lavoué, R., J. J. Van Der Lugt, M. J. Day, M. Georges, V. Busoni, A. C. Merveille and D. Peeters (2010) Progressive juvenile glomerulonephropathy in 16 related French Mastiff (Bordeaux) dogs. Journal of veterinary internal medicine, **24(2)**: 314-322.

Lee, Y. J., C. C. Chang, J. P. W. Chan, W. L. Hsu, K. W. Lin and M. L. Wong (2011). Prognosis of acute kidney injury in dogs using RIFLE (Risk, Injury, Failure, Loss and End-stage renal failure)-like criteria. *Veterinary Record-English Edition*, ***168*(10),** 264.

Lee, Y. J., Y. Y. Hu, Y. S. Lin, C. T. Chang, F. Y. Lin, M. L. Wong and W. L. Hsu (2012) Urine neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute canine kidney injury. *BMC veterinary research*, ***8*(1)**: 248.

Liao, M. T., C. C. Sung, K. C. Hung, C. C. Wu, L. Lo and K. C. Lu (2012) Insulin resistance in patients with chronic kidney disease. BioMed Research International,

Littman, M. P. (2011) Protein-losing nephropathy in small animals.Veterinary Clinics of North America: Small Animal Practice, **41(1)**: 31-62.

Lucre, V. M., D. F. Kelly, P. DARKER and C. J. Gaskell (1980) Chronic renal failure in young dogs–possible renal dysplasia. Journal of Small Animal Practice, **21(3)**: 169-181.

McDonough, P. L. (2001) Leptospirosis in dogs-current status. *Recent Advances in Canine Infectious Diseases. International Veterinary Information Service (www. ivis. org), Ithaca, document*, (A0112), 0701.

Macdougall, D. F., T. Cook, A. P. Steward and V. Cattell (1986) Canine chronic renal disease: prevalence and types of glomerulonephritis in the dog. Kidney international, **29(6)**: 1144-1151.

Martensson, J., and R. Bellomo (2014). The rise and fall of NGAL in acute kidney injury. *Blood purification*, ***37*(4),** 304-310.

Martínez-Castelao, A., J. L. Górriz, J. Segura-De La Morena, J. Cebollada, J. Escalada, E. Esmatjes and J. L. Llisterri-Caro (2014) Consensus document for the detection and management of chronic kidney disease. *Nefrologia*, ***34*(2)**: 243-62.

Mehta, R. L., J. A. Kellum, S. V. Shah, B. A. Molitoris, C. Ronco, D. G. Warnock and A. Levin (2007) Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Critical care, **11(2)**: R31.

Miller, N. L. and J. E. Lingeman (2007) Management of kidney stones Bmj,**334(7591)**: 468-472.

Miyamoto, T., S. Wakizaka., S. Matsuyama., E. Baba., F. Ohashi., M. Kuwamura., J. Yamate and T. Kotani (1997) A Control of a Golden Retriever with Renal Dysplasia. J. *Vet. Med. Sci.* **59(10):** 939–942.

Morgan, M. S. and M. S. Pearle (2016) "Medical management of renal stones.". BMJ (Clinical research ed.). **352**: i52. PMID 26977089.

Moon, S. J., H. B. Park, S. Y. Yoon and S. C. Lee (2013) Urinary biomarkers for early detection of recovery in patients with acute kidney injury. *Journal of Korean medical science*, ***28*(8)**: 1181-1186.

Mrudula, V., T. V. George, C. Balachandran and B. M. Manohar (2005) Bacteriological and Histopathological study of canine nephritis on clinical samples. J Animal Vet Advances, **4**: 954-958.

Mulders, P.F., A.H. Brouwers, C.A. Hulsbergen-van der Kaa, E.N. van Lin, S. Osanto and P.H. de Mulder (2008) *"[Guideline 'Renal cell carcinoma']". Ned TijdschrGeneeskd.* ***152 (7)****: 376–80.*

Nguyen, M. T. and P. Devarajan (2008) Biomarkers for the early detection of acute kidney injury. *Pediatric nephrology*, ***23*****(12)**: 2151.

Oburai, L. N., V. Rao and B. R. Naik (2015) Clinical And Nephrosonographic Findings In Canine Chronic Renal Failure: A Prospective Study. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8,Issue 6 Ver. II (Jun. 2015), PP. 11-16 www.iosrjournals.org*

Oishi, A., H. Sakamoto and R. Shimizu (1995) Canine plasma erythropoietin levels in 124 cases of anemia. *The Journal of veterinary medical science the Japanese Society of Veterinary Science*, ***57*****(4)**, 747–749.

Oishi, A., H. Sakamoto, R. Shimizu, F. Ohashi and A. Takeuchi, (1993) Evaluation of erythropoietin production in dogs with reduced functional renal tissue. Journal of Veterinary Medical Science, **55(4)**: 543-548.

Petejova, N. and A. Martinek, (2013) Acute kidney injury following acute pancreatitis: A review. *Biomedical Papers*, ***157*****(2)**: 105-113.

Picut, C. A. and B. A. Valentine (1985) Renal fibroma in four dogs. *Veterinary pathology*, ***22*****(4)**, 422-423.

Picut, C. A. and R. M. Lewis, (1987[a]) Juvenile renal disease in the Doberman Pinscher: ultrastructural changes of the glomerular basement membrane. Journal of comparative pathology, **97(5)**: 587-596.

Picut, C. A. and R. M. Lewis (1987[b]) Comparative pathology of canine hereditary nephropathies: an interpretive review. Veterinary research communications, **11(6)**: 561-581

Polzin D. C., C. A. Osborne and S. Ross (2005) Chronic Kidney Disease. In: Ettinger, S. J. and E. C. Feldman. Ed:Textbook of Veterinary Internal Medicine, 6th Ed. Elsevier Saunders, Philadelphia, 1756-1785.

Polzin, D. J. (2011) Chronic kidney disease in small animals.*Veterinary Clinics: Small Animal Practice*, *41*(1), 15-30.

Portman, R. J., J. M. Kissane and A. M. Robson (1986). Use of beta 2 microglobulin to diagnose tubulo-interstitial renal lesions in children.*Kidney International*, *30*(1), 91–8. https://doi.org/10.1038/ki.1986.156

Ramos-Vara, J. A., M. A. Miller, J. L. Ojeda, R. Reid, D. Craft and G. L. Watson (2004) Glomerulocystic kidney disease in a Belgian Malinois dog: an ultrastructural, immunohistochemical, and lectin-binding study.

Randers, E., H. Kristensen, E. J. Erlandsen and S. Danielsen (1998) Serum cystatin C as a marker of the. *Scandinavian* renal function *journal of clinical and laboratory investigation*, ***58*(7)**: 585-592.

Rawdon, T. G. (2001) Juvenile nephropathy in a Samoyed bitch. Journal of Small Animal Practice, **42(5)**: 235-238.

Robertson, J. L. (1986) Spontaneous renal disease in dogs. Toxicologic pathology, **14(1)**: 101-108.

Robertson, J. and M. A. Seguin (2008) Renal Disease: case based approach to acute renal failure, chronic renal failure and protein losing nephropathy.

Sangle, N. (2014) Membranous glomerulonephritis. PathologyOutlines.com website. http://www.pathologyoutlines.com/topic/kidneymemgn.html. Accessed June 15th, 2018.

Schinstock, C. A., M. H. Semret, S. J. Wagner, T. M. Borland, S.C. Bryant, K.B. Kashani and J.C. Lieske (2012) Urinalysis is more specific and urinary neutrophil gelatinase-associated lipocalin is more sensitive for early detection of acute kidney injury. *Nephrology Dialysis Transplantation*, ***28*(5):** 1175-1185.

Segev, G., L. D. Cowgill, S. Jessen, A. Berkowitz, C. F. Mohr and I. Aroch, (2012) Renal Amyloidosis in Dogs: A Retrospective Study of 91 Cases with Comparison of the Disease between SharPei and NonSharPei Dogs. Journal of veterinary internal medicine, **26(2)**: 259-268.

Segev, G., C. Palm, B. LeRoy, L. D. Cowgill and J. L. Westropp (2013) Evaluation of neutrophil gelatinase associated lipocalin as a marker of kidney injury in dogs. Journal of Veterinary Internal Medicine, **27(6)**: 1362-1367.

Senior, D. F., J. M. Gaskin, S. A. Hines, C. D. Buergelt, J. W. Harvey and T. J. Keefe (1986). A model for experimental bacterial cystitis in the dog. *Laboratory animal science*, ***36*(5),** 486-491.

Sharma, N. and M. N. Shrestha (2011) Biochemical Analysis for the Determination of Renal and Pancreatic Disorders in Dogs. Nepal Journal of Science and Technology, **12**, 61-68.

Shaw D.H. and S.L. Ihle (2013) Small animal internal medicine. John Wiley and Sons.

Snedecor, G. W. and Cochran, W. G. (2004) Statistical Methods: 7th Edition The Lowa state University Prass, Ames, Lowa.

Sosnar, M., P. Kohout, M. Ruzicka and L. Vrbasova, (2003) Retrospective Study of Renal Failure in Dogs and Cats Admitted to University of Veterinary and Pharmaceutical Sciences, Brno During 1999-2001. *ActaVeterinaria Brno*, ***72*(4)**, 593-598.

Srinivasan, S. R., T. S. S. Rajan, P. Dhanapalan, M. Thanikachalam and V. Gnanaprakasam (1993) Evaluation of certain routine laboratory tests in the diagnosis of renal insufficiency in canine. Ind. J. Vet. Med., **13** : 58-61

Sperschneider, H. (1977) Pathophysiology and therapy of anemia in chronic renal insufficiency. *Zeitschrift fur die gesamte innere Medizin und ihre Grenzgebiete*, ***32*(16)**, 385–388.

Steinbach, S., J. Weis, A. Schweighauser, T. Francey and R. Neiger, (2014) Plasma and Urine Neutrophil Gelatinase–Associated Lipocalin (NGAL) in Dogs with Acute Kidney Injury or Chronic Kidney Disease. Journal of veterinary internal medicine, **28(2)**: 264-269.

Stokes J.E. and J. Bartes (2006) Causes of acute renal failure. CompendContinEducPract Vet **28(5)**: 387–496

Tietz, N. W. (1986) Textbook of Clinical Chemistry, 2nd ed^{n}., W.B. Saunders Co., Philadelphia, 18-19.

Tilley L P and F W K Smith (2007), "Renal Failure",Blackwell's Five Minute Veterinary Consult: Canine and Feline, pp. 1186-1189, Blackwell Publishing, USA.

Tufani, N. A., J. L. Singh, M. Kumar, D. Gupta, P. Shekhar and V. S. Rajora, (2015). Renal failure in Indian dogs: An epidemiological study. *Indian J. Vet. Med. Vol*, ***35*(1),** 7-11.

Vaden, S. L., J. Levine and E. B. Breitschwerdt (1997) A retrospective case control of acute renal failure in 99 dogs. Journal of Veterinary Internal Medicine, **11(2)**: 58-64.

Vegad, J. L. (2005) Textbook of Veterinary General Pathology. 1st ed^{n}. Vikas Publishing House Pvt. Ltd., India : 5.

Wakamatsu, N., K. Surdyk, K. P. Carmichael and C. A. Brown (2007) Histologic and ultrastructural studies of juvenile onset renal disease in four Rottweiler dogs. Veterinary pathology, **44(1)**: 96-100.

Weise, M., D. Prufer, G. Jaques, M. Keller and A. W. Mondorf, (1981) β2-Microglobulin and Other Proteins as Parameter for Tubular Function1. In *Experimental and Clinical Aspects of Proteinuria* **(Vol. 24, pp. 88-98)** Karger Publishers.

Wilcock, B.P. and J. M. Patterson (1979) Familial glomerulonephritis in Doberman pinscher dogs. The Canadian Veterinary Journal, **20(9)**: 244.

Wright, N. G., E. W. Fisher, W. I. Morrison, W. B. Thomson and A. S. Nash, (1976) Chronic renal failure in dogs: a comparative clinical and morphological study of chronic glomerulonephritis and chronic interstitial nephritis. The Veterinary record, **98(15)**: 288-293.

Yang, J., D. Goetz, J. Y. Li, W. Wang, K. Mori, D. Setlik and J. Barasch (2002) An iron delivery pathway mediated by a lipocalin. *Molecular cell*, ***10*(5)**: 1045-1056.

Yhee, J. Y., C. H. Yu, J. H. Kim, K. S. Im, S. K. Chon and J. H. Sur (2010) Histopathological retrospective study of canine renal disease in Korea, 2003~ 2008. Journal of veterinary science, **11(4)**: 277-283.

Yilmaz, A., E. Sevketoglu, A. Gedikbasi, S. Karyagar, A. Kiyak, M. Mulazimoglu and S. Hatipoglu (2009) Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *Pediatric Nephrology*, ***24*(12)**: 2387.

Zeng, X., D. Hossain, D. G. Bostwick, G. A. Herrera and P. L. Zhang (2014). Urinary β2-microglobulin is a good indicator of proximal tubule injury: a correlative study with renal biopsies. *Journal of biomarkers*, *2014*.

APPENDIX

Table 1 : Normal range of hematological parameters in dogs

Parameters	Range
Hb (g/dl)	12 -18
PCV (%)	37- 55
TEC (10^6/cumm)	5.5 - 8.5
MCV (fl)	60 -77
MCH (pg)	19.5 - 24.5
MCHC (g/dl)	32 - 36
TLC (10^3 / cumm)	6 – 17
N (%)	60 – 77
L (%)	12 - 30
PLT (10^3 / cumm)	2 - 5

Table 2 : Normal ranges of biochemical parameters in dogs

Parameters	Range
SGPT (IU/L)	21 -102
SGOT(IU/L)	23 - 66
ALP(IU/L)	20 -156
TP (g/dl)	5.4 - 7.1
ALB (g/dl)	2.3 - 3.3
GLB (g/dl)	2.4 - 4.6
TB (mg/dl)	0.1- 1.0
DB (mg/dl)	0 - 0.25
IB (mg/dl)	0 - 0.75
BUN (mg/dl)	10 -28
CREAT (mg/dl)	0.5- 1.5

THESIS ABSTRACT

1	**Title of the thesis (in Capital letters)**	**:**	CLINICOPATHOLOGICAL STUDIES OF CANINE RENAL DYSFUNCTION WITH SPECIAL REFERENCE TO LIPOCALIN AND BETA 2-MICROGLOBULIN BIOMARKERS
2	**Full name of student**	**:**	LOTHE KALYANI MADHUKARRAO
3	**Name and address of Major Advisor**	**:**	Dr. D. P. Kadam Professor, Department of Veterinary Pathology Bombay Veterinary College, Parel, Mumbai – 400 012
4	**Degree to be awarded**	**:**	M.V.Sc.
5	**Year of award of degree**	**:**	2018
6	**Major subject**	**:**	Veterinary Pathology
7	**Total number of pages in the thesis**	**:**	
8	**Number of words in the abstract**	**:**	
9	**Signature of Student**	**:**	
10	**Signature, Name and address of forwarding authority (HOD / SH)**	**:**	
11	**Signature of the Associate Dean**	**:**	

ABSTRACT

This study was undertaken to study incidence and prevalence of renal disorders in dogs as well as to explore the potential of lipocalin and β2 microglobulin biomarkers for early detection of renal dysfunction. Departmental survey of canine serum biochemical samples over a 2-year period (2015-2017) was carried out and cases having serum creatinine value more than 1.5 mg/dl were considered for survey. Fifty clinical cases of renal disorders (serum creatinine value > 2 mg/dl) were identified over a period of 8 months (February 2017-September 2017) and detailed hematological, serum biochemical and urine examinations were carried out. Information regarding age, sex and breed were noted. Three postmortems of dogs renal disorders cases were selected for this study. Detailed postmortem examination was carried out. Twenty-five (25) departmental necropsy cases were included for histopathological examination.

Sample survey of departmental data revealed that the highest incidence of renal disorders was recorded in the age group of 6-10 years (66.67%) followed by 0-5 years (22.22%). Males were affected more than females. Common breeds found to be affected were Labradors (29.62%) followed by non-descript breeds (25.92%).

The clinical case study of 50 samples reported that the highest incidence of renal disorders was recorded in the age group of 6-10 years (52.27%; ARF(73.33%) and CRF(26.67%)), followed by 0-5 years of age (34.1%; ARF(56.52%) and CRF(43.48%)). Males were affected more than females. Common breeds found to be affected were Labradors (40.9%) and Pomeranian dogs (15.9%) followed by Nondescript (13.63) and German Shepherd (9.09%).

There was significant decrease in the values of Hb, PCV, TEC mainly in CRF cases with 40% of cases showing leukocytosis. Serum creatinine value was increased in dogs which are affected with renal dysfunction.

Fifty samples were processed for lipocalin (NGAL) by ELISA (BIOPORTO). There was statistically significant difference in the values of NGAL within the groups ($P \leq 0.05$). Dogs with AKI had the highest serum NGAL concentration when compared with CKD and healthy groups, whereas dogs with CKD had higher concentration of serum NGAL than that of healthy group.

Amongst these 50 samples, 14 CRF samples were processed for B2M by ELFA (mini VIDAS) for presence of amyloidosis and glomerular damage. The result for B2M was negative as all the 14 values were in normal range (0.3 to 3.0 mg/l).

Out of 3 clinical necropsy cases of canine renal disorders, 1 case was diagnosed as membranous glomerulonephritis and 2 cases were diagnoses as interstitial nephritis. Among departmental necropsy cases 15 cases were diagnosed as interstitial nephritis (60%), 2 cases were diagnosed as glomerulonephritis (8%), in 1 case (4%) calcification observed and in remaining cases degenerative and necrotic changes has been observed. Macroscopically, small granular contracted kidneys were seen in most of the cases, while microscopically lesions were primarily evident in interstitial spaces and tubules.

To conclude, it was found that renal disorders were seen in all age groups but dogs of age 6 - 10 years were mostly affected. Males were more prone to renal disorders than females and incidence of renal disorders was more in Labradors and Pomeranian. Dogs with AKI had the highest serum NGAL concentration when compared with CKD and healthy groups. Therefore serum NGAL biomarker could be useful for detection of AKI in dogs.

प्रबंध सारांश

1	प्रबंधाचे नाव	:	लायपोकॅलीन आणि बिटा 2 मायक्रोग्लोबुलीन जैवचिन्हकांच्या विशेष संदर्भासह श्वानांतील मुत्रपिंड बाधेचा चिकित्साविकृतीशास्त्रीय अभ्यास
2	विद्यार्थ्याचे नांव	:	लोथे कल्याणी मधुकरराव
3	मार्गदर्शकाचे नाव	:	डॉ. डी.पी. कदम, प्राध्यापक, पशु विकृतीशास्त्र विभाग, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई—12
4	पदवी	:	पदव्युत्तर पदवी
5	पदवी प्रदान करण्याचे वर्ष	:	2018
6	मुख्य विषय	:	पशु विकृतीशास्त्र
7	प्रबंधाची एकूण पाने	:	
8	सारांशाचे एकूण पाने	:	
9	विद्यार्थ्याची सही	:	
10	विभाग प्रमुखाचे नाव	:	
11	सहयोगी अधिष्ठाता	:	

प्रबंध सारांश

सदरचा संशोधन प्रकल्प हा श्वानांमधील मुत्रपिंड विकारांच्या घटनांचे प्रमाण, प्रसार आणि लायपोकॅलीन व बिटा 2 मायक्रोग्लोबुलीनचे मुत्रपिंडासंबंधी बिघडलेले कार्य लवकर ओळखण्यासाठीचे संभाव्य शोध लावण्यासाठी करण्यात आला. पशुविकृतीशास्त्र विभागातील 2 वर्षाच्या कालावधीत (2015–2017) रक्तजल क्रिएटीनीनचे प्रमाण 1.5 मि.ग्रॅ./डिएल पेक्षा जास्त असलेल्या श्वानांची नोंद घेण्यात आली. 8 महिन्यांच्या कालावधीत (फेब्रुवारी 2017 ते सप्टेंबर 2017) मुत्रपिंडाचा विकार असलेले (रक्तजल क्रिएटीनीन मुल्य > 2.2 मि.ग्रॅ./डिएल) 50 श्वान निवडण्यात आले आणि त्यांचे तपशीलवार रक्तपरिक्षण, रक्तजल यांची नोंद घेण्यात आली. मुत्रपिंड विकार असलेल्या 3 श्वानांचे शवविच्छेदन करून त्यांच्या अभ्यास करण्यात आला. हिस्टोपॅथालाजीकल परिक्षांसाठी 25 विभागीय शवविच्छेदनांचे समाविष्ट करण्यात आले.

विभागीय आकडेवारीचा नमुना सर्वेक्षणानुसार मुत्रपिंड विकार 6–10 वर्षे वयोगटात (66.67%) होते, त्यानंतर 0–5 वर्षे वयोगटात (22.22%) होते. नर श्वानांमध्ये हे प्रमाण जास्त होते तर लॅब्रॉडॉर (29.62%), त्यानंतर गावठी श्वान (25.92%) या जातीच्या श्वानांमध्ये जास्त प्रमाणात मुत्रपिंड विकार आढळले.

50 श्वानांच्या क्लिनीकल केस अभ्यासात असे आढळले की, मुत्रपिंडाचा विकार 6–10 वर्षे वयोगटात (52.27%), त्यानंतर 0–5 वर्षे वयोगटात (34.1%) होते. नर श्वानांमध्ये हे प्रमाण जास्त होते तर लॅब्राडॉर (40.9%), पॉमेरॅनियन (15.9%), गावठी श्वान (13.63%) आणि जर्मन शेफर्ड (9.09%) या जातीच्या श्वानांमध्ये जास्त प्रमाणात मुत्रपिंड विकार आढळले.

एचबी, पीसीव्ही आणि टिईसीची घट प्रामुख्याने सीआरएफच्या प्रकरणांमध्ये झाली असून 40 टक्के प्रकरणांमध्ये ल्युकोसायटारेसीस आढळून आले. मुत्रपिंड विकार असलेल्या श्वानांमध्ये रक्तजल क्रिएटीनीनचे मुल्य वाढलेले होते.

एलिझा (बॉयाप्रोटेा) व्दारे लॉयपोकॅलीन (एनजीएएल) साठी 50 रक्तजल नमुने तपासण्यात आले. गटांमधील एनजीएएल मुल्यांमध्ये सांख्यकीयदृष्टया

लक्षणीय फरक होता. (पी> 0.05). सीकेडी आणि निरोगी गटाच्या तुलनेत एकेआईच्या श्वानांमधला रक्तजल एनजीएएलचा प्रमाण जास्त होता.

या 50 रक्तजल नमुन्यांपैकी अमॉलॉयडोसीस आणि ग्लोमेरूलर हानी उपस्थितीसाठी ELFA (मिनी VIDAS) व्दारे बी2 एमसाठी 14सीकेडीचे नमुने घेण्यात आले. बी2 एमसाठीचा परिणाम नकारात्मक होता कारण 14 मुल्ये सामान्य श्रेणीत होती (0.3 ते 3.0 मि.ग्रॅ./एल).

3 क्लिनीकल केसेसेच्या श्वानांच्या शवविच्छेदनाअंती असे आढळले की 1 श्वानाला मेंबरेनस ग्लोमेरूलर मुत्रपिंड दाह तर 2 श्वानांना इंदरस्टीशीयल मुत्रपिंड दाह झाला होता.

विभागीय शवविच्छेदनातील 15 प्रकरणे इंदरस्टियल मुत्रपिंड दाह, 2 प्रकरणे ग्लोमेरूलर मुत्रपिंडदाह, 1 प्रकरणात (4%) कॅल्सीफिकेशन आढळले आणि उर्वरीत प्रकरणांमध्ये डिजनरेटीव व नेक्रोटीक बदल आढळून आले. अधिकतर श्वानांचे मुत्रपिंड आकाराने लहान आकसलेले व खडबडीत आढळले व सुक्ष्मदर्शकीय अभ्यासानुसार व्याधी या इंदरस्टीशीयल आणि टयुबुल्समध्ये आढळल्या.

या अभ्यासाअंती असा निष्कर्ष काढण्यात आला की सर्व वयोगटातील श्वानांमध्ये मुत्रपिंड विकार आढळतो मात्र 6–10 या वयोगटात यांचे प्रमाण जास्त आढळते. नर श्वानांमध्ये हे प्रमाण जास्त असते तसेच लॅब्राडॉर आणि पॉमेरॅनीयम श्वानांमध्ये मुत्रपिंड विकार जास्त प्रमाणात आढळतात. एकेआईच्या श्वानांमध्ये रक्तजल एनजीएएलचे प्रमाण सर्वाधिक आढळले. त्यामुळे रक्तजल एनजीएएलचा जैवचिन्हकांचा श्वानांमध्ये एकेआईचा शोधासाठी उपयुक्त ठरेल.

VITA

The author Dr. Kalyani Madhukarrao Lothe was born on 14th April 1992, at Arattondi, taluka Arjuni Morgaon, Gondiya District of Maharashtra state.

She completed her primary education in Jilha Parishad Kendra Prathmik School, Chamorshi and secondary education in Shivaji Highschool, Chamorshi, and passed the S.S.C. examination in distinction with 85.05% in 2008. She completed her H.S.C. education with 70.00% from Nevjabai Hitkarini Mahavidyalaya, Bramhapuri in 2010.

Being interested in animal welfare, she has joined Nagpur Veterinary College, Nagpur in 2010 and completed the B.V.Sc. and A.H. with first class in 2015. She has actively participated in college cultural programmes & represented her university in Indradhanushya Youth Festival which was held at Nanded in 2013. She has completed her course work for Masters Degree in Veterinary Pathology from Bombay Veterinary College, Mumbai, under MAFSU, Nagpur with 8.5 CGPA.

During these two fruitful years in the department she has assisted many research projects in the department. She has actively participated and presented many abstract, posters and cases in different national and international conferences and seminars.