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Gurwinder Singh

Department of Veterinary
Medicine, Guru Angad Dev
Veterinary and Animal Sciences
University, Ludhiana, Punjab,
India

Sikh Tejinder Singh

Department of Veterinary
Medicine, Guru Angad Dev
Veterinary and Animal Sciences
University, Ludhiana, Punjab,
India

Ashwani Kumar Sharma

Department of Veterinary
Medicine, Guru Angad Dev
Veterinary and Animal Sciences
University, Ludhiana, Punjab,
India

Kuldeep Gupta

Department of Veterinary
Pathology, Guru Angad Dev
Veterinary and Animal Sciences
University, Ludhiana, Punjab,
India

Corresponding Author:

Gurwinder Singh

Department of Veterinary
Medicine, Guru Angad Dev
Veterinary and Animal Sciences
University, Ludhiana, Punjab,
India

Study on histopathological findings in dogs with different stages of renal insufficiency

Gurwinder Singh, Sikh Tejinder Singh, Ashwani Kumar Sharma and Kuldeep Gupta

Abstract

The current study involved 50 dogs irrespective of gender, all displaying primary symptoms of azotemia. Each dog underwent an extensive clinical assessment, hematological and biochemical analysis and urinalysis. Radiographic and ultrasonographic assessments were done in 23 and 34 dogs respectively. Based on the structural abnormalities observed in the ultrasonographic scans, various renal conditions associated with chronic renal failure (CRF) were identified in the majority of the dogs. Renal biopsies were conducted on the seven dogs suffering from the advanced stage of renal insufficiency using ultrasound guidance. The obtained samples underwent histopathological analysis to investigate the underlying reasons and origins of kidney conditions. Chronic interstitial nephritis was identified in four cases, involving various dog breeds: a Bull Mastiff, a German Shepherd, a Pomeranian, and an American Bully. All dogs diagnosed with CRF received conservative medical treatment to manage clinical signs of uremia, maintain fluid, electrolyte, and acid/base balance, provide adequate nutrition, and minimize the progression of renal failure.

Keywords: Renal biopsy, ultrasound, renal failure, dogs

1. Introduction

The urinary system particularly the kidneys play an important role in eliminating metabolic wastes from body, maintaining acid-base balance and production of hormones like renin, cholecalciferol and erythropoietin. Kidneys excrete waste products of protein metabolism through urine and hence regulate fluid, electrolyte and acid-base homeostasis (Sebastian *et al.*, 2007) [7]. Renal and extra renal diseases affect the health and functioning of the kidneys significantly thus lead to renal failure. Renal damage may result from infectious, immune-mediated, congenital, metabolic, neoplastic, traumatic and obstructive disease processes. Additionally, several factors such as renal ischemia or nephrotoxic substances result in renal damage resulting in acute or chronic kidney failure. Chronic kidney disease (CKD) is defined as the presence of functional or structural changes in one or both kidneys for greater than 3 months. It is the most common kidney disease in small animals, with an estimated prevalence of up to 7% in dogs. Chronic kidney disease affects the dogs of all ages, but the disease is more common in older dogs. The disease is typically a progressive illness, resulting in significant morbidity and mortality in dogs (McGrotty, 2008) [4]. Tufani *et al.* (2015) [9] documented that the incidence of canine renal failure in India was 2.58%, with the most substantial rates occurring in Patna (6.82%), followed by Pantnagar (5.48%), and Jabalpur (0.58%). Diagnosis of renal failure is based on routine urinalysis, haemato-biochemical and electrolyte assay (Squires, 2005) [8]. Ultrasonography, in contrast, is a non-invasive and highly valuable method for identifying and characterizing renal parenchymal features (Kumar *et al.*, 2011) [2]. A renal biopsy is frequently necessary to confirm a conclusive diagnosis and assess the extent of the kidney lesion. An exact and precise histological diagnosis may also be essential to create the most effective treatment strategy. To gauge the effectiveness of therapy, it's crucial to have a thorough understanding of both the disease's type and its severity (Minkus *et al.*, 1994) [5].

2. Materials and Methods: Out of total 50 cases with renal insufficiency, renal biopsy was

performed in 7 cases (as per requirement). The ultrasound guided procedure was done with animal under sedation using Butorphanol @ 0.2-0.4 mg/kg body weight. The dog was positioned in dorsal recumbency, and the area over the kidney was clipped and surgically prepared. The ultrasound machine was then used to locate the kidney and identify a suitable biopsy site. The Bard Max-Core Tru-Cut 18 G biopsy gun was used to obtain tissue samples. The gun features a spring-loaded needle that is quickly and easily advanced through tissue to obtain a core sample. Once the biopsy was complete, the dog was then monitored closely for any signs of complications, such as bleeding or pain. Suitable pain management medication was provided as and when needed. The tissue samples obtained were collected in 10% formalin solution and subsequently taken for histo-pathological examination.

Procedure

Collection and fixation

The container was labelled with patient information and date. The kidney tissue biopsy specimen was immersed in 10% neutral buffered formalin immediately.

Transport and fixation

- Specimen was taken to the laboratory without delay to prevent tissue degradation.
- The specimen was allowed to fix in formalin for a minimum of 6-24 hours. The exact duration varied based on the size of the specimen and the specific requirements of the laboratory.

Specimen processing

- After fixation, the kidney tissue specimen was transferred into a processing cassette for further processing.
- The cassette with the specimen was placed into a tissue processing machine. The machine dehydrates, clears, and infiltrates the tissue with paraffin wax. This process usually takes several hours.

Embedding

- Once the tissue was adequately infiltrated with paraffin wax, it was removed from the processing machine.
- The tissue block was embedded in a fresh block of paraffin wax using a mould.

Microtomy

- Embedded tissue block was sectioned into thin slices (usually 3-5 micrometres thick) using a microtome. These slices are known as tissue sections or slides.
- Sections were made to float in a warm water bath to flatten and stretch them.

Mounting on slides

- Each tissue section was lifted carefully using a glass slide and water.
- The water was allowed to evaporate; leaving the tissue section adhered to the slide.

Staining

Haematoxylin and eosin (H&E) stain was used for staining the tissue sections following proper staining protocols and timing instructions.

Cover slipping

A coverslip was applied to each stained tissue section using a

mounting medium to protect the specimen.

Microscopic examination

The stained tissue sections were examined under a microscope at 10X, 40X and 100X to record observations and make a diagnosis.

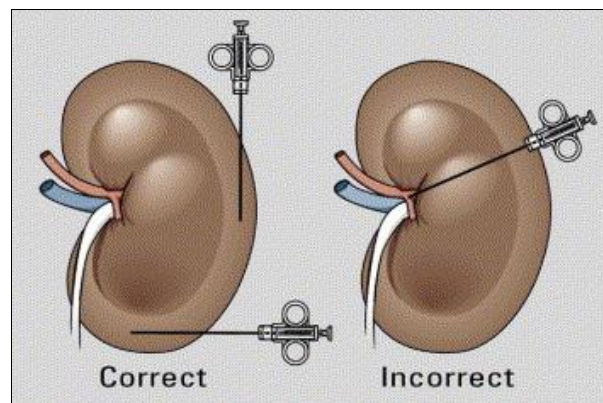


Fig 1: Diagram illustrating the appropriate and inappropriate techniques for guiding the renal biopsy needle. It is important to keep the needle within the renal cortex, preferably at either the top or bottom pole. Avoid crossing the corticomedullary junction or entering the renal medulla or pelvis (Vaden, 2004) ^[10].

Diagram illustrating the appropriate and inappropriate techniques for guiding the renal biopsy needle. It is important to keep the needle within the renal cortex, preferably at either the top or bottom pole. Avoid crossing the corticomedullary junction or entering the renal medulla or pelvis (Vaden, 2004) ^[10].

3. Results

Renal biopsies were conducted on the seven dogs suffering from the advanced stage of renal failure using ultrasound guidance. The obtained samples underwent histopathological analysis to investigate the underlying reasons and origins of kidney conditions. Chronic interstitial nephritis was identified in four cases, involving various dog breeds: a Bull Mastiff, a German Shepherd, a Pomeranian, and an American Bully. Additionally, glomerulosclerosis was diagnosed in a German Shepherd dog. Chronic glomerulonephritis classified as membrano-proliferative glomerulonephritis was observed in two cases, one of which was a non-descript breed, and the other case was a Labrador. Renal biopsy is frequently necessary to establish a definitive diagnosis and evaluate the extent of the lesion. A precise and accurate histological diagnosis is also essential to develop an effective treatment strategy. To assess the response to therapy accurately, it is crucial to have an understanding of the type and severity of the disease being treated (Grauer *et al.*, 1983; Richards *et al.*, 1994; Vilafranca *et al.*, 1994) ^[1, 6, 11]. The results of the renal biopsy were consistent regardless of the age and breed of the dogs, as there was no discernible correlation between the various types of nephropathies and the age of the animals. Similarly, Minkus *et al.* (1994) ^[5] reported that there was no significant association between different types of nephropathies and age, and the animals with chronic tubulo-interstitial nephritis tended to be, on average, older than the animals with glomerulopathies.

Chronic interstitial nephritis was characterized by interstitial fibrosis, tubular atrophy, and the infiltration of interstitial inflammatory cells, primarily lymphocytes and plasma cells. Within the renal corpuscles, there were contracted glomeruli

with an increased volume of mesangial matrix and a thickened Bowman's capsule. This condition progresses with the scarring of the tubulo-interstitium, leading to tubular atrophy. Additionally, there was infiltration of mononuclear cells into the interstitium and tubules.

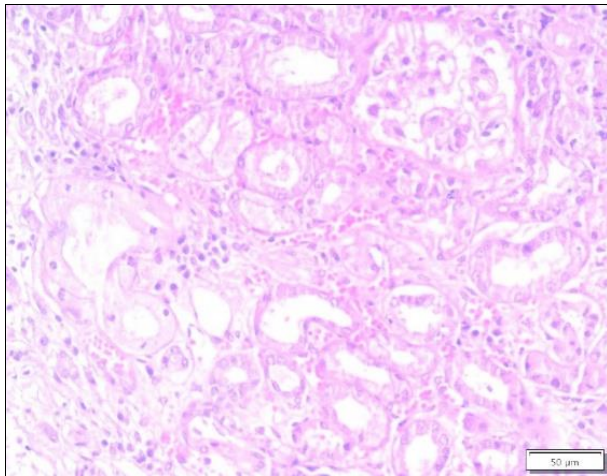


Fig 2: Chronic interstitial nephritis in a dog

Chronic interstitial nephritis

- Characterized by interstitial fibrosis, tubular atrophy and interstitial inflammatory cells (lymphocytes and plasma cells) infiltration.
- The renal corpuscles have contracted glomeruli with increased volume of mesangial matrix and thickened Bowman's capsule

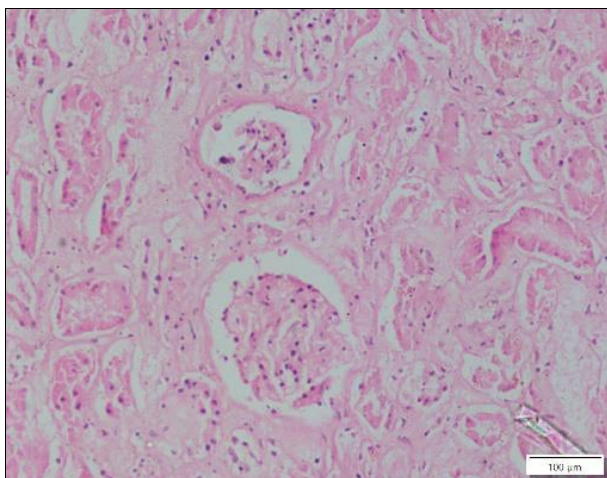


Fig 3: Glomerulosclerosis in a dog

Glomerulosclerosis

Table 1: Histopathologic findings in dogs with renal insufficiency

S. No.	Breed	Age (years)	ALT (U/L)	BUN (mg/dL)	Creatinine (mg/dL)	Histopathological findings
1	Bull mastiff	6.5	32	282	24.6	Chronic interstitial nephritis
2	German Shepherd	10	25	174	11.6	Glomerulosclerosis
3	Non-Descript	12	16	140	15.5	Membrano-proliferative glomerulitis
4	German Shepherd	10	11	130	15.8	Chronic Interstitial nephritis
5	Labrador	8	23	155	11.9	Membrano-proliferative glomerulitis
6	Pomeranian	13	31	164	17.2	Chronic Interstitial nephritis
7	American bully	6.5	39	134	17.6	Chronic Interstitial nephritis

4. Conclusion

In dogs and cats with glomerular disease or acute renal failure, renal biopsy is often recommended as a diagnostic and

- There is hypercellularity, shrinkage and increased fibrous connective tissue and mesangial matrix and almost complete loss of glomerular capillaries
- This is the end stage of chronic glomerulonephritis and the glomeruli are non-functional

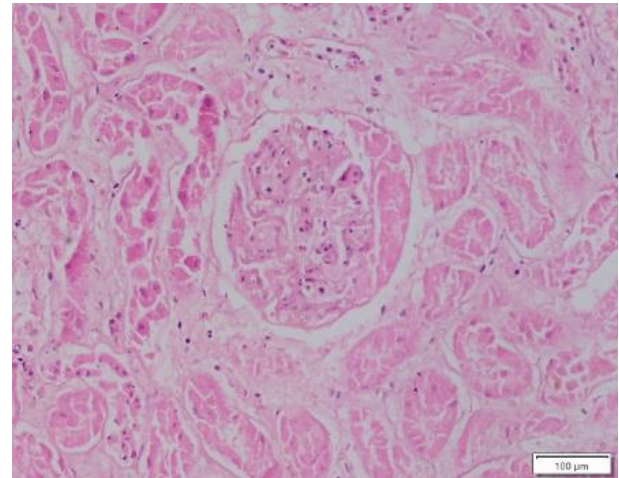


Fig 4: Chronic glomerulonephritis and interstitial nephritis in a dog

Chronic glomerulonephritis and interstitial nephritis

Loss of cells, nuclei and thickening of basement membrane
Increased interstitium as compared to tubules, and replaced by mature fibrous connective tissue

Glomerulo-sclerosis was characterized by several histological changes in the glomeruli including hypercellularity, shrinkage of the glomerular structure, an increase in fibrous connective tissue, and thickening of the mesangial matrix. In advanced stages, there was almost complete loss of glomerular capillaries. This condition represented the end stage of chronic glomerulonephritis, and at this point, the glomeruli were non-functional, and they had lost their ability to filter blood effectively. In cases of chronic glomerulitis there was a reduction in the number of cells and nuclei within the affected glomeruli and renal tubules. The basement membrane, which envelops the glomeruli and tubules, might exhibit abnormal thickening. In membrano-proliferative glomerulitis the interstitium, the space between tubules and glomeruli, tended to expand in volume relative to the tubules. This increased interstitial space was often replaced by mature fibrous connective tissue. Consequently, the presence of this fibrous tissue lead to the replacement of the normal functional components of renal tissue, ultimately contributing to a loss of renal function (McGavin & Zachary 2006) [3].

management tool. This procedure can be performed in these animals through either percutaneous or surgical methods. It is crucial to ensure the use of proper technique. When

performed correctly, renal biopsy is a relatively safe procedure that has minimal impact on renal function, provided patient factors are appropriately considered. Post biopsy monitoring is essential to detect severe haemorrhage, which is the most common complication. To ensure an accurate diagnosis of glomerular disease and effective treatment planning, it is necessary to evaluate the biopsy specimens not only through light microscopy with special stains but also via electron and immunofluorescent microscopy.

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