Bacteriological and Histopathological Study of Canine Nephritis on Clinical Samples

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Abstract: The study on canine nephritis was carried out 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 of nephritis suggested that renal diseases were not diagnosed in the early stages because of the less sensitivity of routinely used screening tests like BUN and serum creatinine.

Key words: Bacteriology, histopathology, canine nephritis

INTRODUCTION

Renal diseases are important clinical problems encountered in dogs and are frequent causes for illness and death. Although renal disorders are more common in older dogs, recent reports show that dogs of any age could be affected. It may affect glomeruli, tubules, interstitial tissue and / or vessels. Some renal diseases may be associated with dysfunction (example: nephrogenic diabetes mellitus) or biochemical abnormalities (cystinuria) without detectable morphological abnormalities. Others may be associated with morphologic renal disease that affects one or both kidneys. Due to the reserve capacity of the kidneys, early renal disease may have only a few consequences to the animal. Renal disease may regress, persist, or advance. Unfortunately many renal diseases are not detected until they become generalised, leading to serious impairment of renal function. The condition may progress to renal failure due to late diagnosis of kidney dysfunction. Final stage renal failure is associated with a high rate of fatality and a high financial expenditure for therapy. Hence this study has been carried out to study the bacteriological and histopathological features of canine nephritis on clinical samples.

MATERIALS AND METHODS

Experimental animals: The study on canine nephritis was carried out 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 diagnosis was confirmed by serum biochemical and ultrasonographical studies. The mean age of affected animals was 7.8 ± 0.34 years. Sixty per cent of the animals affected were males and 40% were females. Aged dogs

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Fig. 2: Biochemical test of E. Coli (isolated from urine sample)
A. OF test: Indicative of sugar utilization fermentatively
B. TSI slant: Acid slant and acid butt with gas production
C. IMVC test, left to right (1 to 4) Indole +ve; MR+ve; VP-ve; citrate -ve
(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.

Isolation of bacteria: The urine samples collected from the suspected cases were inoculated into nutrient broth and incubated at 37°C for 24 h. The cultures were plated onto nutrient agar and incubated at 37°C for 24 to 48 h. Selective media like EMB agar, Mac Conkey agar and Mannitol salt agar (HiMedia, India) were used for isolation of different organisms. The colony characters of the isolated organisms on the above said media are given (Fig 1).

Identification of bacteria: Smears were made from the pure cultures and stained by Grams method. The cultures were plated on Urichrome II agar plates (International Microbio, France, Fig. 1) for rapid identification and the results were interpreted according to manufacturer’s instructions. Biochemical tests like Oxidation Fermentation (OF) test, Indole, Methyl Red (MR), Voges Proskauer (VP), Citrate Utilization test and Triple Sugar Iron (TSI) test were conducted (Fig. 2).

Gross pathology: Renal lesions observed at autopsy in 26 dogs out of the 60 clinical cases as well as from 30 dogs submitted to the department for postmortem examination were included for pathological study. Detailed postmortem examination was conducted and gross lesions were recorded (Fig. 3).

Fig. 3: Gross lesions in Kidney
A. Glomerulonephritis, pin head sized greyish necrotic foci and pitting of cortex
B. Acute interstitial nephritis enlarged kidney with scattered pin sized whitish foci
C. Chronic interstitial nephritis pale and shrunken kidney with granular pitted cortex
D. Nephrolithiasis

Histopathology of the specimen: Tissue pieces for histopathological study collected in 10% formalin were routinely processed and embedded in paraffin. Tissue sections of 5 micron thickness were cut and stained by Haematoxylin and Eosin (H and E). Sections were also stained by periodic acid schiff (PAS), Von Kossa’s method, pearl’s stain and Warthin-Starry method for spirochaetes (Fig. 4).

RESULTS AND DISCUSSION

Urine culture: Among 38 samples (out of 60) cultured, 10 (26.32%) revealed bacterial organisms. Six out of 10 cultures (60 %) yielded Escherichia coli and it was reported to be the most common bacteria causing urinary tract infections in dogs and 3 yielded Staphylococcus aureus in pure culture and one was a mixed culture of E.coli and S.aureus. McCaw et al. reported that bacteriuria and pyuria indicated urinary tract infections but did not localize the infection to kidneys.

Pathology: Out of the 60 clinical cases studied 26 were followed up to post mortem. Thirty cases that showed gross lesions in kidney at routine necropsy which either died or euthanized due to other causes were also included in this study. The types of nephritis encountered were classified according to histopathological findings. Out of 56 cases studied 25 animals (12.5%) showed glomerulonephritis, 47 (83.92%) tubulo interstitial nephritis and 2 (3.57%) embolic nephritis. Out of 7 glomerulonephritis, 4 were subacute membranous glomerulonephritis and 3 were chronic glomerulonephritis. Out of the 47 cases of tubulo interstitial nephritis, 4
animals (8.16%) showed acute tubulointerstitial nephritis, 10 (21.27%) subacute tubulointerstitial nephritis and 33 (70.21%) chronic tubulointerstitial nephritis. Ten animals with tubulointerstitial nephritis were positive for leptospirosis. Out of 56 cases, 36 (67.86%) showed diffuse and 18 (32.15%) focal changes.

**Gross pathology:** Kidneys were normal in size in subacute membranous glomerulonephritis whereas reduced in size in chronic glomerulonephritis. Kidneys were pale in colour. Capsule was adherent to the cortical surface which showed pitting and was granular in appearance. Murray et al.,[7]and Wright et al.,[8] reported similar gross lesions in chronic glomerulonephritis.

In acute interstitial nephritis, kidneys appeared either normal or enlarged in size. Capsule was slightly adherent to the cortex. Scattered pinhead sized whitish foci were present on the cortical surface which extended into the parenchyma on incision. Kidneys were dark red in colour in one case whereas pale in 3 cases. Scattered petechiae were present on the cortical surface. In subacute interstitial nephritis kidneys were slightly enlarged in size. Capsule was moderately adherent to the cortex. Cortical surface was granular and pitted in appearance. Kidneys were firm in consistency and pale in colour. In case of chronic interstitial nephritis kidneys were small in size and contracted. Capsule was firmly adherent to the cortex and thickened. Cortical surface was pale, shrunken and pitted with granular appearance. Scattered irregular grayish white foci were present on the cortical surface. Cysts of varying size (2.5mm) were present on the cortical surface. Kidneys were hard to cut. Corticomedullary junction was congested. In two animals the renal pelvis was dilated and contained yellowish, irregular shaped nephrolith.

**Histopathology:** Based on histopathology, glomerulonephritis was classified into 2 categories. In subacute membranous glomerulonephritis, glomeruli showed thickening of capillary basement membrane with PAS positive material. Two cases showed glomerular haemorrhage. Some glomeruli showed partial to complete loss of tuft. Presence of 'epithelial crescents' was observed in the glomeruli. This agreed with the findings of Koeman et al.,[9] who observed diffuse uniform thickening of the capillary wall without cellular proliferation and epithelial crescent formation. Secondary changes present in the tubules included degeneration and necrosis of tubular epithelium. Focal to diffuse infiltration of plasma cells and lymphocytes were observed in the interstitium and around the glomeruli. Chronic glomerulonephritis was characterised by glomerular sclerosis and obliteration as observed earlier by[10]. Thickening of Bowman's capsule and periglomerular fibrosis were present. Glomerular tuft showed varying
Fig. 5: Kidney showing presence of leptospira organism in the interstitium (warthin-starry staining X 1000)

degrees of atrophic changes and mineralisation. Tubules showed atrophic changes in some areas with severe interstitial fibrosis. Focal infiltration of plasma cells was also noticed.

Tubulointerstitial nephritis was classified into three types. In acute tubulointerstitial nephritis, kidneys showed scattered infiltration of predominantly polymorphonuclear cells in the interstitium as well as in the lumen of tubules. Interstitial edema was also noticed. Tubular epithelium showed degenerative and necrotic changes. Glomerular capillaries and interstitial vessels were congested. Haemorrhage into the interstitium and tubules were also noticed in one case. Similar observations were made by[1,2]. Subacute tubulointerstitial nephritis was characterised by multifocal infiltration of inflammatory cells predominantly lymphocytes and plasma cells in the interstitium. Tubular epithelium showed varying degrees of degenerative and necrotic changes with calcification. Interstitial vessels were prominent with mild interstitial fibrosis. Tubular stasis and mild interstitial edema were present. Presence of golden yellow pigment within the tubular epithelial cells was noticed which was positive with peri-staining. Glomeruli showed atrophic changes in the tuft and thickening of the Bowman's capsule. Chronic tubulointerstitial nephritis revealed extensive infiltration of inflammatory cells especially lymphocytes and plasma cells throughout the interstitium of the kidney. Fibroplasia was extensive and seen extending from cortex to medulla. Tubules showed cystic distension with flattening of tubular epithelium and presence of cellular debris in the lumen. Some of the tubular epithelial cells showed hyperplastic changes. Tubular epithelium showed varying degrees of degeneration, necrosis and calcification. Thickening of tubular basement membrane and peri-tubular fibrosis were observed in some cases.

In leptospirosis cases, leptospiral organisms could be demonstrated by Warthin-Starry method (Fig. 5). Changes noticed in glomeruli were striking. Glomeruli showed thickening of Bowman's capsule with varying degrees of atrophy of tuft. In some glomeruli the tuft was replaced by homogenous eosinophilic filtrate.

Periglomerular fibrosis was present. Some glomeruli were completely obliterated. Blood vessels showed marked congestion. There was thickening of blood vessel walls with narrowing of lumen and mild perivascular fibrosis. This showed the highest incidence of 70.21%.

In case of Embolic nephritis, the glomeruli were completely filled with eosinophilic mass surrounded by infiltration of neutrophils, eosinophils, lymphocytes and plasma cells. In some of the glomeruli clumps of bacterial organism could be discovered. Scattered areas of suppuration with massive infiltration of neutrophils were seen. In another case an attempt for organization was seen with mild fibroplasia around the glomeruli and infiltrating mononuclear cells. E. auereus could be isolated from one case, whereas no organism could be isolated from the other. Dunning and Stonehewer[4,5] indicated antimicrobial therapy already under way as the probable cause of failure to isolate bacterial organisms on culture.

CONCLUSIONS

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 of nephritis in this study suggested that renal diseases were not diagnosed in the early stages because of the less sensitivity of routinely used screening tests like BUN and serum creatinine. A mortality of 43.3% was observed and histopathologically most of them showed chronic interstitial nephritis.

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REFERENCES


