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EFFECT OF VARIOUS RENAL BIOPSY TECHNIQUES ON HAEMATO-BIOCHEMICAL PARAMETERS IN DOGS

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ABSTRACT

Eighteen mongrel dogs of either sex were divided into three equal groups of six animals each, and were subjected to three different renal biopsy techniques viz., ultrasound-guided biopsy using 14-G Tru-cut biopsy needle (Group A), ultrasound-guided fine-needle aspiration biopsy (Group B) and laparoscope-guided biopsy using laparoscopic biopsy forceps (Group C) to study the changes in various haemato-biochemical parameters. The estimations were made just before and at 24, 48 and 72 h post-biopsy. Haematological parameters (Hb, PCV, TLC and DLC) and serum protein remained within the normal reference range in all groups throughout the study period and did not show any significant alterations at any observation interval within and amongst the groups. In all groups, serum creatinine and urea nitrogen increased at 24 h post-biopsy, started to decrease thereafter and by the end of study period the values reached nearer to their corresponding base values. Serum sodium values showed significant ($P < 0.05$) decrease only at 48 h post-biopsy interval in the animals of group A and it was also significantly ($P < 0.05$) different from corresponding value in group C. Serum sodium values showed non-significant variation throughout the study period in the animals of group B and C. The group B values were significantly different from corresponding values of other groups at all post-biopsy intervals.

Keywords: Aspiration, biopsy, laparoscope, ultrasound

INTRODUCTION

In mammalian body kidneys are valuable organs contributing in flushing away different metabolites from blood circulation that are synthesized by various body systems. These metabolites, if not excreted periodically, may prove detrimental to essential body organs resulting into irreversible pathological changes in them (Patel, 2006). They also maintain water, electrolyte, and acid-base balance in the body (Pratt, 1992; Clark *et al*., 1999). Kidneys do suffer from many kinds of diseases in all species including dogs and cats (Vaden, 2004). Their occurrence increases with the advancement in age. The most common renal affections may have infectious, neoplastic or traumatic cause and these affections may be disseminated throughout the kidney or involve a part of it such as solitary renal cyst and nephroliths (Schepper, 1977). Renal biopsy provides information that may confirm, support or eliminate the diagnostic probabilities formulated on the basis of history, physical examination, laboratory data, diagnostic radiographic or ultrasonographic evaluation (Rezaie *et al.*, 2008). Renal biopsy thus plays a valuable role in determining the specific cause of primary kidney disease (Osborne *et al.*, 1996; Drost *et al.*, 2000). However, the renal biopsy techniques may also be associated with complications. The study

was thus undertaken to assess haemato-biochemical alterations during different renal biopsy techniques in order to determine the safety of these techniques.

MATERIALS AND METHODS

The study was conducted on 18 mongrel dogs of either sex of almost uniform size and weight. The dogs were randomly divided into three equal groups of six animals each. Group A animals were subjected to ultrasound-guided biopsy using 14-G 15 cm long with 2 cm specimen notch Tru-cut biopsy needle (Baxter Healthcare Corp., Valencia, CA, USA). Group B animals were subjected to ultrasound-guided fine-needle aspiration biopsy using 18-G spinal needle mounted on a 10 ml syringe and group C animals were subjected to laparoscope-guided biopsy using laparoscopic biopsy forceps.

For haematological studies whole blood samples were collected from cephalic vein in dry clean test tubes containing anticoagulant (EDTA @ 2 mg ml^{-1} blood). Haemoglobin (Hb), packed cell volume (PCV) and total leucocyte count were estimated with the help of automated haematology analyzer (Haemascreen-18, Hospitax, Italy). However, for differential leukocyte count (DLC), freshly prepared blood smears were stained with Wrights-Giemsa stain and values estimated using standard procedures (Schalm *et al.*, 1975).

For biochemical studies, 5 ml blood samples were collected in dry test tubes without anticoagulant. Serum was separated by centrifugation (@ 2000 rpm for 2 min.) two hours after collection and stored at refrigeration temperature for further use. The biochemical parameters serum creatinine, serum urea nitrogen (SUN), protein, sodium (Na^+) and potassium (K^+) were estimated with the help of semi-automated biochemical analyzer (Erba-chem Pro, Transasia Biomedicals, Serum Ltd., India). The data was statistically analyzed as per the standard procedures (Snedcor and Cochran, 1982).

RESULTS AND DISCUSSION

The base value of haemoglobin, packed cell volume and total leucocyte count in animals of all groups was within normal reference range without any significant difference ($P > 0.05$) among them (Table 1). During the study period, the values remained within the normal reference limits in all groups at all observation intervals. Haemoglobin showed non-significant decrease by 24 or 48 h post-biopsy and started increasing thereafter. Packed cell volume decreased at 24 h post-biopsy in the animals of groups A and C. It increased at 48 h post-biopsy interval till the end of study period where it was still lower than the corresponding base values in both the groups A and C. In group B, PCV values decreased upto 48 h post biopsy. The non-significant and transient decrease in packed volume could be due to transient anaemia following biopsy.

Table 1: Mean value of haematological parameters in different groups at various time intervals

Hematological Parameter	Group	Time (h)			
		0	24	48	72
Haemoglobin (g dL^{-1})	A	9.88±0.60	9.25±0.79	9.36±0.71	9.41±0.65
	B	10.15±0.70	9.75±0.75	9.68±0.74	9.70±0.74
	C	10.36±0.66	10.08±0.74	10.05±0.76	10.31±0.66
Packed cell volume (%)	A	31.36±2.32	28.78±2.18	29.33±2.38	29.43±2.42
	B	29.20±1.74	27.63±1.52	27.46±1.10	28.11±1.13
	C	30.71±1.74	30.05±1.02	30.30±1.71	30.41±1.67
Total leucocyte count (x 10^3 μL^{-1})	A	10.69±0.58	12.40±0.64	11.57±0.62	11.11±0.62
	B	10.09±0.94	12.20±0.65	10.98±0.66	10.65±0.64
	C	11.05±0.98	12.17±1.01	11.67±1.10	11.40±0.47

In the animals of all groups the total leucocyte and differential leucocyte count was within normal reference range before and after the biopsy procedures. TLC increased from base value at 24 h post-biopsy interval, started decreasing at 48 h and followed the same trend till the end of study period in all the groups.A non-significant decline in DLC was observed in the mean values of monocytes and eosinophils at 24 h post-biopsy. The values remained stationary thereafter till the end of the study period where they were slightly lower than their corresponding base values (Table 2). Comparison within and among the groups did not show any significant difference (P >0.05) in these parameters at any observation interval, thereby revealing that all these biopsy techniques have minimal effect on haematological parameters. These findings are in total agreement with those of Patel *et al*. (2007) and Rezaie *et al*. (2008). Groman *et al*. (2004) also reported no change in the haematological parameters following serial USG-guide renal biopsy in adolescent dogs.

Table 2: Differential leucocyte count in different groups at different time intervals

Time (h)	Leucocytes	Group A	Group B	Group C
0	Neutrophils	66.16±4.01	66.66±4.92	66.50±6.34
	Lymphocytes	22.66±2.96	22.83±1.86	23.33±2.56
	Monocytes	6.33±0.67	6.16±0.35	6.00±0.45
	Eosinophils	4.83±0.45	4.33±0.56	4.00±0.55
	Basophils	0.16±0.02	0.00±0.01	0.16±0.45
24	Neutrophils	68.66±3.52	68.66±4.95	68.33±6.65
	Lymphocytes	23.50±3.00	23.66±3.50	24.83±3.59
	Monocytes	4.50±0.28	4.66±0.25	4.71±0.45
	Eosinophils	3.33±0.35	3.00±0.25	2.99±0.34
	Basophils	0.00±0.05	0.00±0.09	0.00±0.20
48	Neutrophils	68.50±3.95	68.66±4.54	69.33±4.95
	Lymphocytes	23.50±2.56	23.66±3.00	23.83±3.54
	Monocytes	4.66±0.60	5.00±0.70	4.33±0.75
	Eosinophils	3.33±0.50	3.06±0.45	3.00±0.55
	Basophils	0.00±0.04	0.00±0.07	0.00±0.21
72	Neutrophils	67.50±4.90	67.83±5.60	69.00±6.28
	Lymphocytes	22.50±2.69	23.16±3.54	23.33±3.99
	Monocytes	5.66±0.72	5.33±0.76	4.50±0.79
	Eosinophils	4.33±0.45	3.83±0.52	3.16±0.59
	Basophils	0.00±0.05	0.00±0.09	0.00±0.24

The base value of serum protein in all groups was within normal reference range without showing any significant difference (P > 0.05) among them. In all the groups, the serum protein showed a decreasing trend from base value at 24 h post-biopsy. It started increasing at 48 h post-biopsy and continued with the same trend till the end of study period. However, these changes were non-significant and the values remained within normal reference range during the study (Table 3).

Evaluation of glomerular function is an essential part of the diagnostic approach to patients with suspected renal disease because Glomerular Filtration Rate (GFR) is directly related to functional renal mass. Serum creatinine and blood urea nitrogen concentrations are commonly used screening tests of glomerular function. Creatinine is the waste product of creatine, which is involved in muscle contraction. The concentration of creatinine in blood increases mainly because of excretory dysfunction and renal damage. Its concentration in blood is not influenced by diet; therefore, creatinine levels in blood could be a better prognostic indicator of renal function as compared to urea (Kelly, 1984). To have a significant rise in serum creatinine value, the GFR must fall to about half its normal value and when the concentration of creatinine exceeds 2.0 mg dL^{-1} then only it is said that GFR is reduced. The normal serum creatinine value in dog is about 0.3- 1.3 mg dL^{-1} (Dibartola, 1995). The base value of

serum creatinine in all groups was within normal reference range without showing any significant difference ($P > 0.05$) among them. However, the base value was somewhat higher in group C than other groups. In the animals of all groups, the serum creatinine increased from base value at 24 h post-biopsy (Table 3). Thereafter the creatinine values tended to decrease so as to reach its base value. This could be attributed to the fact that renal function might have returned to its normal level at the end of study period. Comparison within the groups did not reveal any significant ($P > 0.05$) difference except at 24 h post-biopsy interval from the corresponding base value in the animals of group A. The greater increase in creatinine concentration at 24 h post-biopsy might have been due to decrease in renal cortical perfusion and hence the GFR to a greater extent. However, comparison among the groups did not reveal any significant difference ($P > 0.05$) in the corresponding values at different corresponding intervals throughout the observation period. Renal excretion of urea occurs by glomerular filtration and blood urea nitrogen (BUN) concentrations are inversely proportional to GFR. Urea absorption is a passive process. As the fluid passes along the tubule, water is absorbed. The urea concentration rises and so diffuses out of the tubule along the concentration gradient into the peritubular capillaries. As the urea is excreted by glomerular filtration, approximately 25 to 40% of the amount filtered is reabsorbed during its passage through the tubule. High rates of urine flow diminish tubular reabsorption of urea (Osborne *et al*., 1972). In the animals of all groups, the BUN concentration increased from base value at 24 h post-biopsy. The BUN concentration then tended to reach its base value which could be attributed to the fact that renal function might have returned to its normal level. Comparison within the groups revealed significant difference ($P <0.05$) only at 24 h post-biopsy interval from the corresponding base value in the animals of group A and this value was significantly ($P <0.05$) higher than that of group C at the corresponding interval. The greater increase in BUN concentration might have been due to decrease in the GFR to a greater extent caused by injury to the blood vessels of kidney due to the Tru-cut needle.

Table 3: Mean value of biochemical parameters in different groups at various time intervals

Parameters	Group	Time (h)			
		0	24	48	72
Serum protein ($g\ dl^{-1}$)	A	5.67 ± 0.11^{Aa}	5.30 ± 0.25^{Aa}	5.35 ± 0.14^{Aa}	5.58 ± 0.14^{Aa}
	B	5.42 ± 0.30^{Aa}	5.22 ± 0.28^{Aa}	5.35 ± 0.25^{Aa}	5.54 ± 0.23^{Aa}
	C	5.74 ± 0.23^{Aa}	5.61 ± 0.20^{Aa}	5.77 ± 0.21^{Aa}	5.63 ± 0.18^{Aa}
Serum creatinine ($mg\ dl^{-1}$)	A	0.99 ± 0.076^{Aa}	1.28 ± 0.053^{Ab}	1.13 ± 0.044^{Aab}	1.08 ± 0.06^{Aa}
	B	1.11 ± 0.064^{Aa}	1.27 ± 0.043^{Aa}	1.21 ± 0.062^{Aa}	1.16 ± 0.07^{Aa}
	C	1.21 ± 0.087^{Aa}	1.29 ± 0.071^{Aa}	1.26 ± 0.07^{Aa}	1.12 ± 0.062^{Aa}
Blood urea nitrogen ($mg\ dl^{-1}$)	A	25.99 ± 2.29^{Aa}	32.14 ± 1.17^{Ab}	29.98 ± 1.78^{Aab}	27.03 ± 1.90^{Aab}
	B	23.84 ± 2.62^{Aa}	28.08 ± 2.12^{ABa}	26.01 ± 2.76^{Aa}	24.10 ± 2.47^{Aa}
	C	21.39 ± 2.37^{Aa}	23.91 ± 2.48^{Ba}	22.61 ± 2.17^{Aa}	21.89 ± 2.41^{Aa}
Serum sodium ($m\ Eq\ L^{-1}$)	A	147.97 ± 2.49^{Ab}	143.40 ± 2.16^{Aab}	138.52 ± 2.77^{Aa}	142.48 ± 3.05^{Aab}
	B	145.12 ± 2.58^{Aa}	142.52 ± 2.39^{Aa}	144.41 ± 1.70^{ABa}	147.76 ± 3.35^{Aa}
	C	149.69 ± 2.65^{Aa}	148.21 ± 2.69^{Aa}	149.39 ± 2.51^{Ba}	150.27 ± 2.12^{Aa}
Serum potassium ($mEq\ L^{-1}$)	A	3.58 ± 0.007^{Ac}	3.53 ± 0.008^{Ab}	3.46 ± 0.017^{Aa}	3.46 ± 0.017^{Aa}
	B	3.62 ± 0.0076^{Aa}	3.81 ± 0.0065^{Bb}	4.02 ± 0.0076^{Bd}	3.98 ± 0.004^{Bc}
	C	3.57 ± 0.0047^{Ac}	3.51 ± 0.0042^{Ab}	3.45 ± 0.014^{Aa}	3.45 ± 0.015^{Aa}

Superscripted small letters show comparison within group and superscripts capital letters show comparison between groups; Means with different superscripts differ significantly ($P <0.05$) from each other.

The serum electrolytes monitored in the present study were sodium and potassium. Sodium and its associated anions (mainly chloride) account for more than 90% of the solute in the extracellular fluid and the composition of this extracellular fluid is carefully regulated by various mechanisms but especially by the kidneys (Guyton and Hall, 2000). Normal concentration of plasma Na^+ ranges from

140- 160 mEq L^{-1} in dogs (Kolata, 1985). The base value of serum sodium in all groups was within normal reference range without showing any significant difference (P>0.05) among them. However, the base value was slightly higher in group C than other groups (Table 3). In the animals of groups B and C, the non-significant (P>0.05) reduction in the serum sodium at 24 h from their corresponding base values was observed and thereafter revealed a non-significant (P>0.05) increase till the end of study period. Such a change may be due to decreased renal blood flow and renal cortical perfusion, as depicted in the previous parameter. A long term voiding of more diluted urine activates increase in sodium and chloride delivery to the macula densa in the thick ascending limb of loop of Henle. Contrarily, a statistically significant (P<0.05) decrease in the sodium value at 48 h post-biopsy interval was observed in the animals of group A. This marked alteration can be attributed to increased loss of sodium ions along urine. Comparison among the groups revealed significant (P<0.05) difference in sodium values of the animals of groups A and C at 48 h post-biopsy interval. This marked decrease can be attributed to the increased loss of sodium ions along urine.

Potassium plays a vital role in electrolyte balance in the blood along with sodium and calcium ions. Potassium is an important component of intracellular fluid rather than the extracellular fluid. Potassium output is more regulated by the kidneys where whole potassium is filtered by ultrafiltration method by the glomerulus and whole is reabsorbed before reaching the distal tubules (Christie and Rosin, 1985). Potassium is excreted primarily by way of renal secretion. The majority of potassium appears in urine as a result of its secretion from distal nephron tubular cells into the lumen. Potassium secretion in these segments of the nephron is sensitive to tubular flow rates. Rapid urine formation promotes potassium secretion whereas slow urine formation limits its secretion (Polzin *et al*., 1995). The normal reference range for plasma potassium is from 3.5-5.8 mEq L^{-1} in case of dog (Kolata, 1985). The base value of serum potassium in all groups was within normal reference range without showing any significant difference (P>0.05) among them (Table 3). In group B animals, the mean value of serum potassium revealed significant increase (P>0.05) from base value at 24 h post-biopsy interval and then followed the same trend till 48 h pos-biopsy. This transient significant rise in the potassium ion concentration within the blood may be attributed to the fact that there is decrease in the GFR due to reduced cortical perfusion. As a result of which, there is decrease in ultrafiltration of potassium ions and decline in the reabsorption of the ultrafiltrate. However, as the ultimate urine potassium concentration is dependent on the flow rate of the filtrate through the distal tubular nephron (Polzin *et al*., 1995), the reduced flow rate of the ultrafiltrate may have hampered potassium secretion which eventually contributed to marked increase in potassium levels. The hypoxic changes induced by reduced cortical perfusion might have also contributed to the additive effect of reduced potassium secretion within the tubule. However, in the animals of groups A and C, the mean value of serum potassium started decreasing significantly from base value at 24 h post-biopsy interval and continued with the same trend till the end of study period. Comparison among the groups revealed significant higher (P<0.05) serum potassium values in the animals of group B from the corresponding values at all corresponding intervals of the study period from other groups. Significant effect on GFR and hence on the flow rate of filtrate through the distal tubules to a greater extent might have caused significant change in the serum potassium following renal biopsy in the group B.

Conclusions: Haematological parameters (Hb, PCV, TLC, and DLC) and serum protein showed no significant difference at any observation interval within and among groups and these values were within the normal reference limits in all groups. In all the groups, the serum creatinine and blood urea nitrogen increased from base value at 24 h post-biopsy, started decreasing at 48 h post-biopsy and continued till the end of study period where they tended to reach their corresponding base values. In the animals of groups B and C, the serum sodium values showed a non-significant variation throughout the study period. However, a statistically significant (P<0.05) decrease in the sodium value at 48 h post-biopsy interval was observed in the animals of group A. The mean value of serum potassium in all groups

revealed significant variation throughout the study period. The group B values were significantly different ($P<0.05$) from corresponding values in other groups at all post-biopsy intervals.

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