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Comprehensive study of haemato-biochemical, ascitic fluid analysis and ultrasonography in the diagnosis of ascites due to hepatobiliary disorders in dog

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ABSTRACT

Two percent of the dogs with ascites of hepatic insufficiency were recorded. Decline in the values of Hb, PCV, RBCs and increased TLC and prothrombin time were recorded. Hypoproteinemia, hypoalbuminemia with elevated bilirubin and liver enzymes were observed. Abdominal ultrasound revealed anechoic structure with focal and diffused hyper echoic, cirrhosis of liver and gall bladder disorders were recorded. Ascitic fluid was clear/transudate with a few mesothelial cells, lymphocytes, monocytes and neutrophils in most of the ascitic dogs. Decreased serum total protein, albumin and elevated serum ALT, AST, ALP, total bilirubin, prolonged prothrombin time and SAAG was good indicator for diagnosis of ascites due to HBD. Ascitic fluid analysis and serum ascites albumin gradient (SAAG) may be a key indicator for diagnose etiopathogenesis of ascites in dog.

Key words: Abdominal paracentesis, Ascites, Liver, Portal hypertension

Ascites is a pathological accumulation of free fluid within the peritoneal cavity (Hall and German 2005) and it is mostly accompanied with sever chronic liver disease due to portal hypertension, hypoalbuminenia and increased renal sodium and water retention (Rutgers and Biourge 2007). The diagnosis of liver disorders is a challenge to clinicians because clinical signs are often very vague and non-specific, especially in the early stage of hepatobiliary diseases. Because of varied functions of liver, no single test can accurately identify the hepatic disease or its underlying cause hence, a battery of test is required to assess ascites due to hepatobiliary disorder. So, comprehensive study of ascitic fluid, serum biochemical and serum marker is the need of the present scenario for proper diagnosis of ascites due to hepatic damage. Therefore the present study was taken up to detect the haemato-biochemical, ascitic fluid analysis and ultrasonography changes of ascites due to hepatobiliary disorders in dog.

MATERIALS AND METHODS

Dogs having signs of distended abdomen presented at Referral Veterinary Polyclinic (RVP), Indian Veterinary Research Institute, Izatnagar, was taken under study. History of each dog was noted in relation to breed, age, sex, body weight etc. for epidemiological study. The samples of ascitic fluid and venous blood samples were obtained on the same day. Collected serum samples were stored in deep freezer at –20°C for biochemical estimation.

Haematology: Haemoglobin, PCV, total erythrocyte and leukocyte counts and red blood cell indices (MCV, MCH, MCHC).

Serum biochemistry: BUN, creatinine, ALT, AST, ALP, total bilirubin, total protein (TP), albumin, sodium and potassium and prothrombin time (PT).

Ultrasonography: Ultrasonographic examination was carried out with 2.5–5.0 MHz transducers.

Abdominal paracentesis: Abdominal paracentesis was performed on standing and or left lateral recumbency and the surrounding area was clipped and full surgical preparation was done. Site was chosen 2 to 3 cm caudal to the umbilicus and 2 to 3 cm left of the midline. Urinary bladder was emptied before centesis. A 22 or 20 G needle or catheter was inserted at an angle of 45° to the body wall. Fluid was collected directly into the sterile vials with or without EDTA for further analysis (Alleman 2003, Rudolff 2005).

Ascitic fluid analysis: (a) Physical examination: Colour/ turbidity and specific gravity. (b) Microscopic examination: 1. Cell count was done by haemocytometer counting chamber. 2. Cytological examination: Ascitic fluid cytology smear was stained by Giemsa. (c) Biochemical test: Total protein, albumin and serum ascitic albumin gradient (Alleman 2003).

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Serum ascitic albumin gradient (SAAG): It was calculated by subtracting the albumin concentration of the ascitic fluid from the albumin concentration of a serum obtained on the same day (Beg *et al.* 2001).

Statistical analysis: All data were expressed as means±SE. The analyses were performed with SPSS 16.0. The Student's t-test was used to analyze the study parameters.

RESULTS AND DISCUSSION

Incidence: Total number of dogs brought to RVP, IVRI, Izatnagar irrespective of the nature of the diseases were 3,600 during the course of the study (March 2011– 2012). Out of these, 72 dogs (2.0 %) were observed with ascites due to hepatic insufficiency. Spitz dogs (54.2%) had higher incidence followed by Mongrel (16.4%), Labrador Retriever (12.5%), German Shepherd (6.9%), Doberman pinscher (6.9%), Great Dane (1.4%) and Golden Retriever (1.4%). Higher incidence was recorded in male mongrel dogs (75.0%) whereas in female Doberman pinscher (80.0%) showed higher incidence. Most of the ascities were noticed more than 5 years old (33.3%) followed by 4–5 years (15.3%), 1–2 years (13.9%), 2–3 years (12.5%), 3–4 years (12.5%) and less than one year (12.5%) of age. 2–3 years old male (88.9%) had higher incidence whereas in female more than 5 years (66.7%) old dogs. In the present study the overall sex-wise distribution revealed that male dogs (54.2%) had higher incidences of ascites. Most of the ascites dogs were fed with homemade vegetarian diet. Cases of ascites were recorded from time to time by various researchers in different breeds of dog namely Labrador Retriever (James *et al.* 2008), German Shepherd (Nottidge *et al.* 2003), Doberman pinscher (Fuentealba *et al.* 1997) and Great Dane (Raffan *et al.* 2009). Various authors have reported ascites in different age groups, viz. from 16 weeks to 9 years (mean 3.12 years) and from 1 to 13 (mean 5) years (James *et al.* 2008). Ascites is being considered to be a common complication of chronic hepatitis in dogs (Raffan *et al.* 2009) and it could be aggravated by the activation of hepatic stellate cells with subsequent presinusoidal collagen accumulation and fibrosis, that results in sinusoidal occlusion followed by portal hypertension and promote the formation of fluid accumulation (Hall and German 2005).

Clinical signs: All the dogs showed abdominal distension followed by inappetance (69.4%), lethargy (63.9%), pale mucous membrane (45.8%), respiratory dyspnoea (15.3%), pedal oedema (12.5%), diarrhoea (8.3%), vomiting (6.9%), melena (6.9%) and polyuria/polydipsia (4.2%). The clinical signs of hepatobiliary dysfunctions (HBD) are relatively vague and highly variable in nature. The findings of present study were similar to the observations of various authors (Watson and Bunch 2009, Saravanan *et al.* 2012).

Haematology: Highly significant (P<0.001) decrease in Hb, PCV and RBCs count were noticed in ascitic dogs. Significant (P<0.05) increase in TLC, neutrophil and basophil count and decreased lymphocyte count were observed as compared to healthy dogs (Table 1). Liver being the prime organ, involved in the production of erythropoietin and other factors required for erythropoiesis and so the dysfunction of liver may lead to anaemia (Sharma *et al.* 2001). Washabau (2010) observed severe leukocytosis and neutrophilia in dogs with granulomatous hepatitis, hepatic cirrhosis, hepatic abscess and hepatic neoplasia and emphasized the probable causes for significant increase in total leukocytes, neutrophil and basophil counts in the study.

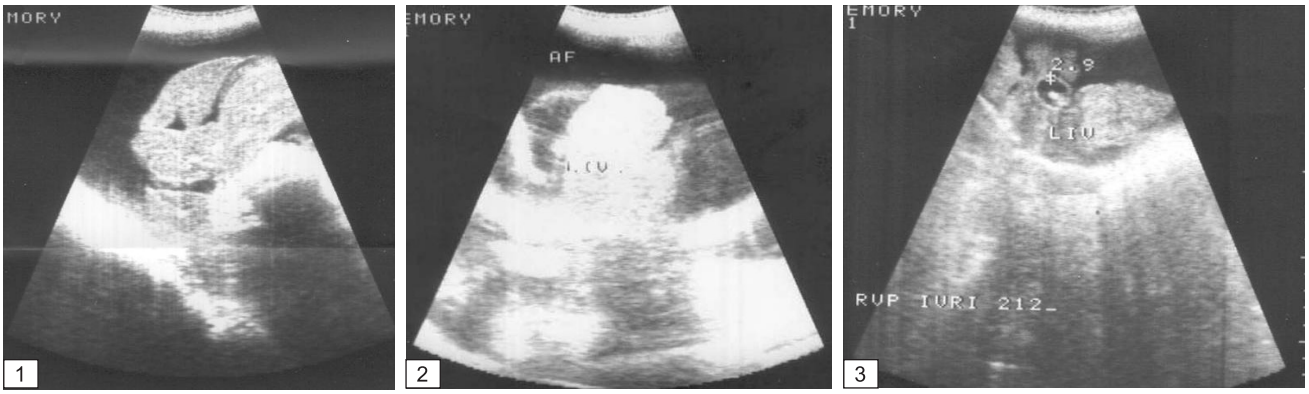
Serum biochemical profile: Significant increase (P<0.05) in ALT, AST, ALP, total bilirubin and prolonged prothrombin time activities were observed in ascitic dogs. Serum AST and ALT measurements were highly useful in detecting hepatocellular injury and monitoring clinical progress (Tennant and Center 2008). Solter *et al.* (1994) stated that the high serum ALP as a sensitive marker for cholestasis in most of the mammalian species including dogs. Highly significant (P<0.001) decrease in total protein and albumin level were noticed in ascitic dogs (Table 2), which could be due to the primary role of liver in the synthesis of major plasma protein as well as site of degradation and synthesis of many other proteins that is influenced by liver diseases in many ways (Webster 2005). Ascites leads to increased albumin distribution and lowers the blood albumin concentration, which decreases the plasma oncotic pressure and aggravates the ascites (Richter

Table 1. Mean±SE of haematological profile in ascitic dogs

Parameters	Control (n=6)	Ascites dog (n=72)
Hb (g/dl)	12.28±0.23	8.90±0.25**
PCV %	43.0±0.83	27.37±0.68**
RBC ($\times10^6$/ /µl)	4.31±0.07	2.73±0.10**
MCV (fl)	100.29±2.95	104.83 ± 2.44^{NS}
MCH (pg)	30.03±0.74	33.73 ± 0.68^{NS}
MCHC (%)	29.97±0.18	32.46 ± 0.36^{NS}
WBC ($\times10^3$/ /µl)	10.39±0.42	18.57±0.87*
Lymphocyte (%)	27.67±1.05	18.50±1.01*
Monocyte (%)	1.67±0.33	1.93 ± 0.13^{NS}
Neutrophil (%)	69.50±0.92	78.19±1.07*
Basophil (%)	0.17±0.17	0.87±0.09*
Eosinophil (%)	1.0±0.26	0.61 ± 0.11^{NS}

Table 2. Mean±SE of serum biochemical profile in ascitic dogs

Parameters	Control (n=6)	Ascites dog (n=72)
BUN (mg/dl)	26.26±1.89	31.29 ± 3.26^{NS}
Creatinine (mg/dl)	0.62±0.15	1.17 ± 0.15^{NS}
Prothrombin time (sec)	5.0±0.25	8.50±0.31*
ALT (IU/L)	41.55±3.37	173.47±12.29*
AST (IU/L)	29.72±2.0	161.18±11.15*
ALP (IU/L)	48.51±2.28	120.74±7.89*
Total protein (g/dl)	6.63±0.09	5.15±0.10**
Albumin (g/dl)	3.31±0.09	2.02±0.03**
Globulin (g/dl)	3.32±0.04	3.13 ± 0.10^{NS}
A: G ratio	1.0±0.03	0.83 ± 0.14^{NS}
Total bilirubin (mg/dl)	0.36±0.03	0.99±0.07*
Na^+ (mEq/L)	136.3±0.8	139.2 ± 1.7^{NS}
K^+ (mEq/L)	4.9±0.1	4.3 ± 0.1^{NS}



Figs. 1–3. **3.** USG: Diffused hyper echoic liver parenchyma with massive ascitic fluid (sagittal view). **2.** USG: Cirrhotic liver parenchyma with massive ascitic fluid (sagittal view). **3.** USG: Cirrhotic liver parenchyma, gall stone (cholelithiosis) with ascitic fluid (sagittal view).

2003, Tennant and Center 2008). Estimation of serum albumin levels and prothrombin time are often considered tests of liver function. These findings are agreeing with the present study.

Ultrasonography: All the 72 dogs were observed with anechoic structure (ascitic fluid) in abdominal cavity followed by 18 dogs (25%) with focal hyper echoic and 33 dogs (45.8%) showed diffused hyper echoic liver parenchyma (Fig. 1) with normal size of liver. Liver cirrhosis (Fig. 2) were found in 19 dogs (26.38%) as evidenced by hyper echoic bright small size liver with irregular margin. Gall bladder disorders (cholecystitis/ cholelithiasis/ distension) (Fig. 3) were noticed in 10 dogs (13.90%). Also multiple hepatic cysts in 1 case (1.4%) and multiple hepatic nodules in 1 case (1.4%) were recorded. In the present study, liver, kidney (right and left), spleen, intestine and urinary bladder were suspended in ascitic fluid during USG examination.The presence of ascitic fluid in the abdominal cavity greatly enhanced the image of various abdominal organs, viz. liver, spleen, intestine, urinary bladder and right and left kidney. Mc Grothy and Doust (2004) stated that the abdominal ultrasound is being considered as more sensitive than survey radiography to detect peritoneal fluid. Cholecystitis as generalized gall bladder wall thickening is associated with acute pyelonephritis, portal hypertension, chronic renal diseases, and hepatitis (Mattoon and Nyland 2002).

Ascitic fluid analysis: Ascitic fluid was aspirated in severely distended/ tensed abdomen to relive respiratory dysponea. Colour/turbidity of ascitic fluid was clear/ transudate in 68 dogs followed by clear/straw colour in 2 dogs and clear reddish yellow in 2 dogs. Cytologically, ascitic fluid revealed a few mesothelial cells, lymphocytes, monocytes and neutrophils (Fig. 4) in most of the ascitic dogs, whereas two dogs showed tumor cells in the ascitic fluid. Specific gravity (1.014±0.001), total nucleated cell count (388.93±66.32/cmm), total protein (1.96±0.08 g/dl), albumin (0.83±0.05 g/dl) and serum ascites albumin gradient (1.20±0.05 g/dl) of ascitic fluid were showed transudative ascites. Paracentesis could assist in timely identifying the pathological causes which are responsible for the fluid accumulation or it can help further investigation to diagnose the diseases condition (Papasouliotis and Dewhurst 2005). The mean specific gravity, total nucleated cell count, total protein, albumin and serum ascites albumin gradient found to be a transudative type of ascites of the present study. These findings are in line with the findings of Burgees (2004).

The SAAG reflects the oncotic pressure gradient between the vascular bed and elevated gradient (greater than or equal to 1.1 g/dl) usually being associated with increased portal pressure, whereas a low gradient (<1.1 g/dl) is associated with conditions where the ascites is not related to portal hypertension (Tarn and Lapworth 2010). Burgees (2004) and Saravanan *et al.* (2012) opined that the serum-ascites albumin gradient (SAAG) provides better discrimination between ascites of chronic liver diseases from other origin. Hence, SAAG > 1.1 g/dl is suggestive of the presence of portal hypertension due to chronic liver disease.

Refractory form of ascites were noticed in 16 dogs (22.22%), of which 3 dogs showed recurrence within a month and the remaining 13 dogs after 1 month from the day of clinical presentation and standard therapeutic intervention. Among these 13 recurrent ascitic dogs, one dog exhibited recurrence of ascites after 6^{th}, 8^{th}, 11^{th}, 13^{th} and 15^{th} month during standard therapeutic intervention. However, 3 dogs exhibited ascites recurrence at 4^{th}, 6^{th}, 8^{th} and 10^{th} month of standard therapeutic intervention. Reoccurrence of ascites in dogs is mostly because of the pet owners discontinuing therapy once after their pets getting clinical aid, response within few day of therapy. Hence, this also could be one of the reasons for recurrence and diuretic resistance ascites which were observed in the present study. Cirrhotic patients with ascites were characterized by marked alterations in their splanchnic and systemic haemodynamics leading to spanchnic congestion, central hypovolaemia and arterial hypertension with activation of rennin-angiotensin and vasoconstrictor systems with renal increase of sodium reabsorption. One of the most adverse consequences of such hemodynamic dysfunctions were the development of refractory ascites in which standard medical treatment with low sodium diet and

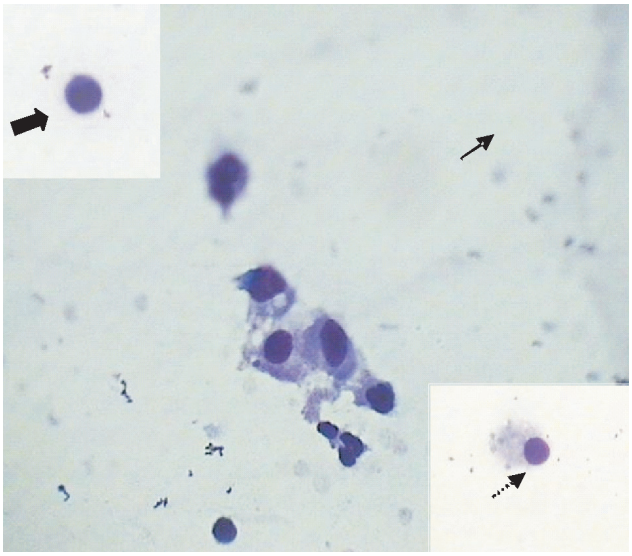


Fig. 4. Ascitic fluid cytology - mesothelial cells, lymphocytes (arrow) and monocyte. Giemsa stain 100×.

diuretics are unable to resolve the ascites. Further massive ascites and hepatorenal syndrome (HRS) are frequent complication of liver cirrhosis (Gerbes and Gulberg 2006). Refractory ascites generally affects with advanced cirrhosis that may also develop HRS. Both the refractory ascites and HRS are the independent predictors of short survival (Salerno *et al.* 2010). Decreased serum total protein, albumin level and increased ALT, AST, ALP, total bilirubin, prothrombin time and serum ascites albumin gradient (SAAG) were good indicator for diagnosis of ascites due to HBD.

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