



RESEARCH ARTICLE

Haematological and Biochemical Studies on Hepatic Disorders in Dogs

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ABSTRACT

Haematological and biochemical tests were made on forty nine dogs suffering from various hepatic disorders. In acute hepatitis, the mean values of Hb, PCV, TEC and platelet count values were decreased non-significantly as compared to healthy control group (n=6). In chronic hepatitis, mean values of Hb, PCV, TEC and platelet count values were decreased significantly as compared to healthy control group. In acute hepatitis, the mean values of ALT, AST and ALP were significantly (P<0.05) increased from that of healthy control group. The values of GGT, bilirubin, creatinine and BUN were found to be non-significantly increased as compared to control group. In chronic hepatitis, the mean values ALT, AST, ALP and BUN were significantly increased as compared to control group. The values of GGT, bilirubin and creatinine were non-significantly increased. In Cholestasis/ cholangiohepatitis, the mean values ALT, AST, ALP, GGT, bilirubin and BUN were found to be significantly increased as compared to control group. Creatinine decreased non-significantly from that of control group. Total protein, albumin, plasma glucose and cholesterol values were decreased significantly whereas globulin and A/G ratio were found to be decreased non-significantly as compared to control group. In the present study cholestasis/cholangiohepatitis (3.08±1.54 mg/dl) showed highest increase in bilirubin levels followed by chronic hepatitis (2.15±0.72 mg/dl) and acute hepatitis (1.9±0.37 mg/dl).

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INTRODUCTION

Liver is the most important vital organ and the largest parenchymal gland of the body with vast reserves of function (70-80%), an almost embryonic capacity to regenerate and perform adequately despite often extensive pathological damage to its integrity. It provides a myriad of biochemical, synthetic, excretory and regulatory functions important to intermediary body metabolism (Center, 1998). There are reports of high incidence of hepatic disorders and one of the top five causes of non accidental deaths in dogs (George *et al.*, 1986). It occurs quite frequently in dogs, the overall incidence in dogs has been estimated around 1-2% of the clinical cases. A wide variety of hepatic disorders encountered in dogs are acute hepatitis, chronic hepatitis and cirrhosis. It plays an important role in maintaining haemostasis, producing pro-coagulant, anti-coagulant and fibrinolytic proteins and also removes clotting factors from the circulation

(Mischke *et al.*, 2003). Hepatic disorders are characterised by decreased values of hemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC), total protein and glucose with increased leucocytes, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), with usually abnormal clotting time (increased), reduced protein synthesis and reduced vitamin K absorption. The current paper recorded haematological and biochemical alterations in clinical cases of various hepatitis in dogs.

MATERIALS AND METHODS

The present study was conducted on the dogs presented in the Small Animal Medicine OPD of Referral Veterinary Hospital of the Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology- Jammu, between June 2010 and August 2011. Dogs with no clinical

condition brought to the clinics for routine clinical examination in the age group of 3-5 years irrespective of sex and breed were chosen to act as control (n=6). All the animals were found clinically, healthy, active and had normal appetite, defecation and urination. Normal clinical parameters, haematology and blood biochemical profiles were obtained from this group. A total of 49 dogs suffering from various hepatic disorders were examined viz: acute hepatitis (n=14), chronic hepatitis (n=23) and Cholestasis/cholangiohepatitis (n=12) on the basis of history, clinical symptoms, haemato-biochemical observations, duration and the progression of the disease diagnosis. After properly restraining the animal, blood samples were collected taking all the aseptic precautions and avoiding haemolysis. About 1 ml of blood was collected in vacutainer containing disodium salt of ethylenediamine-tetra acetic acid (EDTA, 1mg/ml) for haematology and about 4 ml blood was collected in heparinized vacutainer for biochemical estimation and an additional 2 ml of blood was collected in vacutainer containing sodium fluoride as anti coagulant for the estimation of blood glucose. Blood collected in heparin was immediately centrifuged at 3000 rpm for 12 minutes and plasma separated was stored at -20°C till further use. Estimation of Hb, PCV, TEC, TLC and differential leukocytic count (DLC) was done at the methods described by Jain *et al.* (1986). The biochemical estimations viz. ALT, AST, ALP, gama glutamyl transferase (GGT), plasma glucose, plasma cholesterol, total protein, plasma albumin and globulin, A:G ratio, blood urea nitrogen (BUN), creatinine and bilirubin. Biochemical parameters were carried out spectrophotometrically using standard protocols in various reagent kits. Statistical analysis was also done (Snedocor and Cochran, 1967).

RESULTS

In acute hepatitis, the mean values of Hb, PCV, TEC and platelet count were 10.57±0.57 g/dl, 30.85±1.85%, 5.17±0.14 x 10⁶/µl and 3.21±0.12 x 10⁵/µl, respectively and all the values decreased non-significantly as compared to healthy control group. TLC increased to 13.94±0.53 x 10³/µl and this increase was non-significant. Clotting time increased significantly to 2.62±0.09 min. Differential leucocyte count revealed neutrophils, lymphocytes, monocytes, eosinophils and basophils as 71.60±3.20, 24.20±1.60, 3.60±0.29, 0.50±0.14 and 0.10±0.07 per cent respectively. Significant increase in neutrophils was observed as compared to control group.

In chronic hepatitis, mean values of Hb, PCV, TEC and platelet count were 8.29±0.40 g/dl, 26.09±1.36%, 4.53±0.22 x 10⁶/µl and 2.63±0.21 x 10⁵/µl, respectively and all the values were decreased significantly as compared to healthy control group. TLC (14.75±0.92 x 10³/µl) and clotting time (2.51±0.21 min) were increased non-significantly. Differential leucocyte count revealed neutrophils, lymphocytes, monocytes, eosinophils and basophils as 68.36±3.60, 24.79±1.30, 4.51±0.41, 2.13±0.17 and 0.21±0.05 per cent, respectively. Non-significant increase in neutrophils was observed as compared to control. In the Cholestasis/cholangiohepatitis group the mean values of Hb, PCV, TEC and platelet count were 8.13±0.61 g/dl, 26.32±2.05%, 4.37±0.37 x

10⁶/µl and 2.30±0.18 x 10⁵/µl, respectively and all the values decreased significantly as compared to healthy control group. Mean TLC values (14.62±0.82 x 10³/µl) increased non-significantly from that of healthy control. Clotting time increased significantly to 3.19±0.16 minutes. Differential leucocyte count revealed neutrophils, lymphocytes, monocytes, eosinophils and basophils as 64.73±2.80, 27.45±1.90, 4.71±0.27, 2.90±0.21 and 0.19±0.06 per cent respectively. Mild decrease in Neutrophils was observed as compared to control (Table 1).

In acute hepatitis, the mean values of ALT, AST and ALP were found to be 1010.72±180 IU/L, 417.18±17.60 IU/L and 285.12±66.46 IU/L, respectively. Significant increases in these values were observed from that of healthy control group. The values of GGT, bilirubin, creatinine and BUN were 13.49±1.90 IU/L, 1.90±0.37 mg/dl, 1.47±0.17 mg/dl and 22.40±2.19 respectively, these values were found to be non-significantly increased as compared to control group. Plasma glucose, cholesterol, total protein, albumin, globulin, A/G ratio were 83.25±4.40 mg/dl, 198.04±11.43 mg/dl, 6.00±0.19 g/dl, 2.75±0.11 g/dl, 3.21±0.11 g/dl, 0.86±0.04, respectively all these values decreased non-significantly as compared to control group. In chronic hepatitis, the mean values ALT, AST, ALP and BUN were found to be 528.77±17.33 IU/L, 371.39±14.40 IU/L, 405.61±70.02 IU/L and 28.45 mg/dl, respectively and all the parameters were significantly increased as compared to control group. The values of GGT, bilirubin and creatinine were 15.04±2.17 IU/L, 2.15±0.72 mg/dl and 1.54±0.10 mg/dl, respectively and the values were non-significantly increased. Total protein, albumin, globulin, A/G ratio, plasma glucose and cholesterol were 5.17±0.14 g/dl, 2.17±0.07g/dl, 3.00±0.10 g/dl, 0.72±0.03, 63.67±2.10 mg/dl and 117.23±9.27 mg/dl, respectively and were decreased significantly when compared to control group of animals. In Cholestasis/ cholangiohepatitis, the mean values ALT, AST, ALP, GGT, bilirubin and BUN were found to be 431.46±15.70 IU/L, 195.21±21.19 IU/L, 938.19±130 IU/L, 18.25±3.10 IU/L, 3.08±1.54 mg/dl and 26.20±2.51 mg/dl, respectively and all the parameters were significantly increased as compared to control group. Creatinine (0.97±0.10 mg/dl) decreased non-significantly from that of control group. Total protein, albumin, plasma glucose and cholesterol values were 5.62±0.24 g/dl, 2.57±0.12 g/dl, 69.55±4.03 mg/dl and 158.76±8.23 mg/dl, respectively and were decreased significantly from the control group. Globulin and A/G ratio were 3.04±0.19 g/dl and 0.84±0.02, and were found to be decreased non-significantly as compared to control group (Table 2).

DISCUSSION

There was significant fall in Hb, PCV and TEC in chronic hepatitis and cholestasis/cholangiohepatitis while as non-significant decrease was observed in acute hepatitis. Decrease in Hb is attributed to increased degradation of erythrocytes due to increased transit time through spleen because of reduced portal blood flow and or increased fragility of erythrocytes due to high levels of bile acids, besides impaired bone marrow responses, decreased erythrocyte survival time, decreased nutrient uptake due to inappetance or anorexia and reduced

Table 1: Haematological studies in acute, chronic and cholestasis/cholangiohepatitis

S. No	Parameter	Healthy control (n=6)	Acute hepatitis (n=14)	Chronic hepatitis (n=23)	Cholestasis/ Cholangiohepatitis (n=12)
1	Hb (g/dl)	11.95±0.49	10.57±0.57	8.29*±0.40	8.13*±0.61
2	PCV (%)	34.70±2.01	30.85±1.85	26.09*±1.36	26.32*±2.05
3	TEC(x 10 ⁶ /μl)	5.92±0.28	5.17±0.14	4.53*±0.22	4.37*±0.37
4	TLC(x 10 ³ /μl)	11.53±0.78	13.94±0.53	14.75±0.92	14.62±0.82
5	Platelets(x 10 ⁵ /μl)	3.62±0.19	3.21±0.12	2.63*±0.21	2.30*±0.18
6	Clotting time (min)	2.06±0.52	2.62±0.09	2.51*±0.21	3.19*±0.16
7	DLC (%)				
	Neutrophils	65.00±1.71	71.60*±3.20	68.36±3.60	64.73±2.80
	Lymphocytes	25.66±1.54	24.20±1.60	24.79±1.30	27.45±1.90
	Monocytes	5.58±0.31	3.60±0.29	4.51±0.41	4.71±0.27
	Eosinophils	3.25±0.17	0.50*±0.14	2.13±0.17	2.92±0.21
	Basophils	0.16±0.11	0.10±0.07	0.21±0.05	0.19±0.06

*Significant at 5% (P<0.05)

Table 2: Biochemical studies in acute, chronic and cholestasis/cholangiohepatitis

S. No	Parameter	Healthy control (n=6)	Acute hepatitis (n=14)	Chronic hepatitis (n=23)	Cholestasis / Cholangiohepatitis (n=12)
1	ALT (IU/L)	47.25±2.08	1010.72**±180	528.77**±17.33	431.46**±5.70
2	AST (IU/L)	30.22±1.98	417.18**±17.60	371.39*±14.40	195.21*±21.19
3	ALP (IU/L)	64.22±6.60	285.12**±66.46	405.61**±70.02	938.19**±130
4	GGT (IU/L)	8.80±0.52	13.49±1.90	15.04±2.17	18.25*±3.10
5	Glucose (mg/dl)	90.66±2.10	83.25±4.40	63.67**±2.10	69.55**±4.03
6	Cholesterol (mg/dl)	214.22±2.87	198.04±11.43	117.23**±9.27	158.76**±8.23
7	Total protein (g/dl)	6.30±0.05	6.00±0.19	5.17*±0.14	5.62*±0.24
8	Albumin (g/dl)	2.97±0.04	2.75±0.11	2.17*±0.07	2.57*±0.12
9	Globulin (g/dl)	3.35±0.03	3.21±0.11	3.00*±0.10	3.04±0.19
10	A/G ratio	0.88±0.01	0.86±0.04	0.72*±0.03	0.84±0.02
11	Bilirubin (mg/dl)	1.04±0.12	1.90±0.37	2.15±0.72	3.08*±1.54
12	BUN (mg/dl)	20.26±1.84	22.40±2.19	28.45*±2.68	26.20*±2.51
13	Creatinine (mg/dl)	1.35±0.14	1.47±0.17	1.54±0.10	0.97±0.10

*Significant at 5% (P<0.05); ** Significant at 1% (P<0.01)

availability of micronutrients from liver (Bush, 2002). Neutrophilia was observed in all three conditions with significant neutrophilic leucocytosis in acute hepatitis (Stockham and Scott, 2002). Mean platelet count was significantly decreased in all the three groups. Several mechanisms have been suggested for thrombocytopenia in patients with hepatic disorder which include increased platelet sequestration in the spleen as a result of congestive splenomegaly, reduced production of thrombopoietin by the liver, increased platelet breakdown due to antibodies (Prins *et al.*, 2010) and increased consumption resulting from low grade disseminated intravascular coagulopathy. Clotting time was significantly increased in all the three groups, which could be due to improper synthesis of proteins by the liver required for clotting mechanisms. Webster (2005) suggested that liver is the production site for all coagulation factors. Reduced hepatic synthesis results in a clinically significant hypocoagulable state. The activities of ALT, AST and ALP were significantly elevated in acute, chronic hepatitis and cholestasis/cholangiohepatitis. GGT was elevated significantly in cholestasis/cholangiohepatitis and showed a non-significant increase in acute and chronic hepatitis. Elevations of plasma transaminases such as ALT and AST were indicative of altered hepatocellular membrane permeability, hepatocellular necrosis and inflammation with degree proportional to number of injured hepatocytes (Kramer and Hoffman, 1997). ALP is a membrane bound enzyme found on hepatocyte canalicular membrane and luminal surface of biliary epithelial cells and its isoenzymes are

present in kidneys, intestine, placenta and bone, but elevation in its level in more than one year old dog is usually indicative of hepatic origin unless bone disease coexists, since isoenzymes from other organs are having extremely short half lives. Marked increase in activities of ALP and GGT has been reported in conditions causing cholestasis, cholangiohepatitis, biliary cirrhosis, biliary obstruction and cholecystitis of cholelithiasis (Bandyopadhyay, 2003). A significant increase in total bilirubin was observed in the present study and changes were more pronounced in cholestasis/cholangiohepatitis and chronic hepatitis which was in accordance with various authors (Sevelius, 1995; Tiwari *et al.*, 2002).

In the present study cholestasis/cholangiohepatitis (3.08±1.54 mg/dl) showed highest increase in Bilirubin levels followed by chronic hepatitis (2.15±0.72 mg/dl) and acute hepatitis (1.9±0.37 mg/dl). Hyperbilirubinemia is due to disturbance of the balance between rate of production of bilirubin and metabolism and excretion of bilirubin. In the present study the increase might be as a result of diminished excretion due to extensive hepatocyte damage or biliary obstruction or a combination thereof (Vijayakumar *et al.*, 2008). Also a significant decrease in total protein, albumin, globulin level and A/G ratio was observed in chronic hepatitis and cholestasis/cholangiohepatitis where as a non-significant decrease was observed in acute hepatitis. Liver being the main site of synthesis and degradation of most of the proteins, any hepatic disorder (chronic hepatitis and cirrhosis) are responsible for decrease in albumin concentration. Total plasma protein might also have decreased due to marked

decline in the diet intake, malabsorption and ongoing protein losing enteropathies like gastroenteritis, gastrointestinal ulcerations and chronic gastritis. Inflammation also results in increased bowel permeability leading to fluid, electrolyte, protein and cell loss. The findings of the present study in agreement with findings of Sevelius (1995). But hypoalbuminemia may also occur without impairment in hepatic albumin synthesis due to either leakage of albumin from hepatic lymph or increase in volume of distribution as in cases of ascites. Hyperglobulinemia in chronic liver diseases as observed in the present study could be due to increased synthesis of gamma globulin fraction associated with enhanced systemic immune reactivity against portal antigens or secondary to antibody production. The present findings are in agreement with Jacobs and Swan (1995). However in the acute hepatitis, total protein, albumin, globulin and A/G ratio were decreased non-significantly which might be due to short period of duration of disease or short insult to hepatic protein synthesis (Shawn, 2009). Significant decrease in cholesterol level was observed in all the three groups of patients which may be attributed to decrease in synthesis or absorption from the gut or excessive conversion of cholesterol into bile acids (Hall, 1985). There was a significant decrease in the plasma glucose levels which corroborated with the findings of Varshney and Hoque (2002) in dogs with hepatic dysfunctions. Significant hypoglycaemia was observed in chronic hepatitis and cholestasis/cholangiohepatitis where as non-significant decrease in glucose was seen in acute hepatitis. Hypoglycaemia in the affected dogs might be due to inappetance/anorexia complemented by malabsorption from intestine. Hypoglycemia in patients with hepatic disorders results from decreased glycogenolysis and gluconeogenesis combined with hyper-insulinemia due to decreased hepatic metabolism. Blood urea nitrogen increased significantly in chronic hepatitis and cholestasis/cholangiohepatitis and non-significantly increased in acute hepatitis, however the increase was more in chronic hepatitis which corroborates with the findings of Chohan *et al.* (2009). Haemolysis may produce substrates in the form of proteins that would require deamination and consequently lead to hyperammonaemia. Since gastric ulceration has been reported as a major cause of an increased UC ratio in dogs (Prause and Grauer, 1998), it may play a role in the elevated UC ratio encountered in dogs with hepatic disorders. Nine dogs in this study showed typical clinical signs of gastric ulceration. Creatinine levels were non-significantly increased in acute and chronic hepatitis and non-significantly decreased in cholestasis/cholangiohepatitis. Similar observations were made by Chohan *et al.* (2009). This might be due to the renal abnormalities and the urinary retention due to obstruction.

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