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Keywords: serum biochemistry, x-ray, ultrasonography, hepatic problem, dogs and cats.

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Detection of Hepatic Problem in Dogs and Cats by Biochemical Assay and Imaging Technique (X-Ray and Ultrasonography)

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Abstract- Keeping pet (dogs and cats) has become a cultural phenomenon throughout the world including Bangladesh. Hepatic problem is an important health issue that may cause mortality of these pet. This study was conducted with the aim to detect the hepatic problem in dogs and cats along with its frequency in different sex, age and breeds of animals. A total of 100 clinically suspected animals (50 dogs and 50 cats) were included in the study. After thorough clinical examination, 2 ml bloods were collected by venous puncture in serum vials for biochemical analysis. After that all animals were subjected to x-ray and ultrasonography. In biochemical analysis standard deviation of hepatic enzymes (902.48 u/l ALP, 626.66 u/l ALT, 722.98 u/l AST in dog and 564.22 u/l ALP, 300.06 u/l ALT, 340.00 u/l AST in cat) and bilirubin (2.02 mg/dl in dog and 5.22 mg/dl in cat) were remarkably above the mean value (333.86 ± 127.63 u/l ALP, 256.36 ± 88.62 u/l ALT, 261.62 ± 102.24 u/l AST, bilirubin 0.72 ± 0.29 mg/dl in dog and 245.42 ± 79.79 u/l ALP, 180.16 ± 42.43 u/l ALT, 188.99 ± 48.08 u/l AST, 1.81 ± 0.74 mg/dl bilirubin in cat). The standard deviation of other parameters like albumin, total protein, BUN, glucose and cholesterol were below the mean value. Some abnormal morphology (misshaped and enlarged liver) and parenchymal lesions (hypoechoic zone and thickened capsule) were also found in some animals in x-ray and ultrasonography respectively. Hepatic problems were recorded 18% and 12% by serum biochemistry and 6% and 4% by imaging techniques (x-ray and USG) in case of dogs and cats respectively. In age wise distribution, old animals (more than 5 years) (50% in both dog and cats) were more susceptible for hepatic problem that was statistically significant ($P < 0.05$). In conclusion, a good percentage of hepatic problems were identified in both dogs and cats and was more in old animals. So, it is encouraging the pet owner to regular check up their pet in older age to diagnose the hepatic problems in earlier stage. This early diagnosis will be helpful for effective treatment.

Keywords: serum biochemistry, x-ray, ultrasonography, hepatic problem, dogs and cats.

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I. INTRODUCTION

Keeping pet has become a cultural phenomenon in the advanced world. Currently around 13 million of households (48%) own a pet (excluding fish) in the UK (Islam et al., 2013). Dogs (23%) and cats (18%) are the most common pet animals in their houses in Britons currently. In the USA, around 62% of all households have a pet (Pet Industry Market Size and Ownership Statistics, 2013). In terms of number of dogs and cats the figures are around 78.2 million and 86.4 million respectively in the USA. A similar picture has been observed in Europe, Australia, and South East Asia including China, Japan and India. In Bangladesh the pet populations are also increasing day by day. Hypothetically, dog and cat populations in Bangladesh are predicted as 1.6 million and 1 million respectively. This increasing trend of having a pet in the home has been attributed to various reasons including 'misfiring of parental instincts, biophilia (a hypothetical biologically based love of nature), social contagion, the tendency for the middle class to emulate the customs of the rich, the need to dominate the natural world, social isolation in urban societies, and the desire to teach responsibility and kindness to children' (Herzog, 2011). But several health problems of the pet make their owners more tensed and worried. These health problems sometimes may cause mortality in pets. Hepatic problem is one of them. Infectious and inflammatory hepatobiliary diseases are common causes of morbidity and mortality in canine and feline patients (Callahan et al., 2011). The prevalence of the hepatic problems in dog and cat is 1.24% and 0.41% respectively (Apalkova, 2013). The common liver problems in dogs are hepatitis, cirrhosis, bile duct obstruction, gall bladder inflammation, circulatory or blood vessels abnormalities, cancer and toxins (Willard et al., 2012). In case of cat, cholangiohepatitis, hepatic lipidosis and chronic hepatitis are the common problems of liver (Fluen et al., 2019). Liver diseases are considered often to remain undiagnosed. Clinical signs can be absent for a long time until the disease has progressed to a severe stage. Therefore laboratory findings, such as elevated liver enzymes in multiple samples, are what lead to suspect a liver disease more often than clinical signs.

Presence of the liver disease is then usually confirmed with more specific laboratory tests that give clue on liver function (Apalkova, 2013). Although serum biochemistry is considered as important preliminary tools for proceeding toward the correct diagnosis and treatment protocol, radiography is also useful to evaluate the morphologic abnormalities and ultrasonography (USG) is an excellent non-invasive way to evaluate liver parenchyma (Kumar et al., 2012). The literature about biochemical alteration and imaging technique in liver problems is scarce. As per our knowledge, there were no previous published reports on clinical finding and laboratory alterations of liver disorders under Bangladeshi conditions. Hence, the present study was undertaken to detect hepatic problems by assessing several aspects of blood biochemical profile and using imaging techniques (X-ray and USG) in clinically suspected animals. The study was basically focused on only hepatic problems broadly not specific causes or condition in the liver.

II. MATERIALS AND METHODS

a) Ethical statement

The study was carried out with the permission of the director of clinics. The verbal consent from the animal's owner was taken as they visited to the clinics for treatment purpose of the animals. The sample was collected with minimum discomfort of the animal.

b) Study area, period and animals

The present study was conducted through the period of October, 2020 to March, 2021 on 100 animals (50 dogs and 50 cats) that were presented at Teaching and Training Pet Hospital and Research Center of Chattogram Veterinary and Animal Sciences University. Animals of sexes (male and female), different age category like young (upto 1 year), adult (<1 to 5 years) and old (<5 years) and breeds were included in the study. The animals that had signs like anorexia, polyuria (PU), polydipsia (PD), abdominal distension, weight loss, anemia, and jaundice were considered for sample collection.

c) Clinical examination

Each animal was subjected to a detailed clinical examination. Each animal was thoroughly evaluated for its general condition, inspection of mucous membranes, hydration status, signs of pain, and abdominal distension. Information regarding feeding history, polyuria, polydipsia and weight loss were known from the owner by face to face interview.

d) Blood and data collection

Blood sample (2.5 ml) was collected from cephalic vein of each animal using sterile disposable needle. Before collection, the puncture area was cleaned and disinfected with 70% alcohol. The collected blood was stored in serum vials for biochemical

analysis. Data regarding age, sex and breed were collected from hospital case record sheet.

e) Serum biochemistry

After clotting, serum was separated by centrifugation and transferred to a dry clean vial for further evaluation. Humalyzer 3000 semi-automated chemistry system was used to determine the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, total proteins, albumin, blood urea nitrogen (BUN), creatinine, cholesterol, and glucose. The hepatic problems were considered in those animals where the bilirubin, ALP, ALT and AST value were significantly above the reference value.

f) Imaging technique (x-ray and ultrasonography)

All the animals were subjected to chest and abdominal x-ray. After x-ray exposure, abdominal ultrasonography was performed following appropriate procedure. Before USG scanning, the whole ventral abdomen was shaved in each animal. Abnormal morphology (altered size, shape and structure) in x-ray and parenchymal changes in ultrasonography were helped in diagnosis of hepatic problems in some animals.

g) Statistical analysis

All data were stored in excel sheet 2007 and descriptive statistics were calculated in its by using excel sheet software. Frequency distributions were analyzed by using statistical software stata 2017. Significant was considered when p -value <0.05 for chi square test. The occurrences were calculated by dividing total animals with positive case and multiplied by hundred.

III. RESULTS

a) Biochemical analysis findings

The standard deviation were found 2.02 mg/dl (bilirubin), 902.48 u/l (ALP), 626.66 u/l (ALT), 722.98 u/l (AST) for dog and 5.22 mg/dl (bilirubin), 564.22 u/l (ALP), 300.06 u/l (ALT), 340.00 u/l (AST) for cat. The mean values were 0.72 ± 0.29 mg/dl (bilirubin), 333.86 ± 127.63 u/l (ALP), 256.36 ± 88.62 u/l (ALT), 261.62 ± 102.24 u/l (AST) for dog and 1.81 ± 0.74 mg/dl (bilirubin), 245.42 ± 79.79 u/l (ALP), 180.16 ± 42.43 u/l (ALT), 188.99 ± 48.08 u/l (AST) for cat. The higher standard deviation value for bilirubin, ALP, ALT and AST in both dogs and cats indicates that the data above the mean value and were not clustered around the mean. The data showed that there were remarkable changes on bilirubin, ALT, ALP and AST value that indicated hepatic problems in some study animals (Table 3 and 4).

b) *Imaging techniques (x-ray and ultrasonography) findings*

In x-ray and ultrasonography, abnormal structural morphology (misshaped and enlarged liver), abdominal fluid and abnormal parenchymal lesion (hypoechoic foci and thickened capsule with hyperechoic foci) were found in some dogs and cats respectively (Figure 1 and 2).

c) *Occurrence and frequency distribution of hepatic problem in dogs and cats*

The occurrence of hepatic problems in dogs and cats were recorded 18% (9/50) and 12% (6/50) through biochemical analysis respectively. In imaging techniques (x-ray and USG) it was found 6% (3/50) and 4% (2/50) hepatic problems in dogs and cats respectively. The occurrence of hepatic problems was higher in older dogs (50%) and cats (50%) followed by young (0% in dog and 20% in cat) and adult (0% in dog and 5.13% in cat) which is statistically significant ($P<0.05$) (Table 3 and 4). In case of sex, male dogs (22.22%) and cats (20.0%) were more susceptible for hepatic problems than female (13.04% in dogs and 3.85% in cat) (Table 3 and 4). German shepherd dog breeds (42.86%) were more prone to hepatic problems followed by Lash apsu (28.57%), Mixed breed (20.0%) and Local dogs (12.50%) in dogs (Table 3). Local cat breeds (19.05%) were highly susceptible followed by Persian cat (10.53%), Mixed (0%) and Exotic cat (0%) for hepatic problems (Table 4). The hepatic problems were considered in those animals where the bilirubin, ALP, ALT and AST value were significantly above the reference value. Additionally abnormal morphology (altered size, shape and structure) in x-ray and parenchymal changes in ultrasonography were also helped in some animals.

IV. DISCUSSION

Hepatic enzyme assay are performed widely as a popular measurement of the condition of the liver (Watson et al., 2009). These are alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Among these ALT and AST are present higher concentrations in cytosol of hepatocytes and elevated if there is hepatocyte damage. The ALP and GGT enzymes are epithelial, membrane-bound produced from biliary tract in response to certain stimuli, such as cholestasis (Watson et al., 2009). Increased level of these liver enzymes may be a sign of damage to the liver (Center et al., 2007). In our study animal, standard deviation values (902.48 u/l ALP, 626.66 u/l ALT, 722.98 u/l AST in dog and 564.22 u/l ALP, 300.06 u/l ALT, 340.00 u/l AST in cat) of these enzymes were significantly larger than mean values (333.86 ± 127.63 u/l ALP, 256.36 ± 88.62 u/l ALT, 261.62 ± 102.24 u/l AST in dog and 245.42 ± 79.79 u/l ALP, 180.16 ± 42.43 u/l ALT, 188.99 ± 48.08 u/l AST in cat) indicate remarkably

elevation of these enzymes resulting from hepatic problems. These findings were supported by the previous literature. Hyperbilirubinemia is found when the hepatic problem becomes severe stage (Watson et al., 2009). As bilirubin may be normally found in the urine of dogs, very high levels suggest a liver disease. In case of cats bilirubinuria is a clinically important finding (Watson et al., 2009). Standard deviations of bilirubin (2.02 mg/dl in dog and 5.22 mg/dl in cat) were also higher than the mean value (0.72 ± 0.29 mg/dl in dog and 1.81 ± 0.74 mg/dl in cat) in case of both dogs and cats that represents elevation in some animals. As there were changes in the hepatic enzymes value, it might be related to the hepatobiliary problems in both dogs and cats. Decreased albumin and total protein levels may be related to a liver disease, since liver is the only organ producing albumin (Apalkova et al., 2013). Standard deviations of albumin and total protein were below the mean values indicates data points were closed to the average in our study. Liver also metabolizes ammonia to urea, so blood urea nitrogen concentration may fall (Watson et al., 2009). Liver play role in metabolism, its dysfunction may affect numerous other processes and thus change various laboratory findings, such as glucose and cholesterol levels (Watson et al., 2009). Standard deviations value of albumin, total protein, BUN, glucose and cholesterol were below the mean values indicate data points were closed to the average in our study. These findings were not lined with the previous study. These variations may be due to initial stage of hepatic problems or changes of these values were not significant in all hepatic problems diagnosed animal.

Abdominal ultrasonography (US) is the most useful technique for detecting a hepatobiliary disease (Guillot et al., 2009, Feeney et al., 2008). It shows the changes in the parenchymal structure as variation in echogenicity. This helps comparing hepatic tissue to other soft tissues, as well as determining heterogeneous structures within the hepatic parenchyma (Watson et al., 2009). Similarly, some parenchymal lesions like thickened hepatic capsule and hypoechoic lesion (cyst or tumor) in advance stage of hepatic problems were found in this study.

Radiography usually helps in liver diagnostics mostly to detect the size and shape of the liver and possibly to point the lesions more specifically (Watson et al., 2009). Hepatomegaly may be seen as caudal dislocation of gastric axis and pylorus in lateral view of the abdomen (Apalkova et al., 2013). Microhepatia is not as clearly seen, but may be visible as a more perpendicular angle of gastric fundus to the spine in the right lateral projection (Watson et al., 2009). Focal enlargement of one hepatic lobe is shown as dislocation of the organs nearest to the lobe. Agreed with these statements there were some abnormal morphologies

(enlarged and misshaped) of liver were detected in radiology in the current study.

The occurrence of hepatic problems in our study was recorded 18% and 12% in dogs and cats respectively in serum biochemistry. On the other hand, 6% and 4% hepatic problems were recorded in dogs and cats respectively in imaging techniques (x-ray and USG). These observations were so far higher than the observations of some studies where they recorded 1.24% and 0.41% in dogs and cats respectively (Apalkova et al., 2013) and 3.51% in dogs only (Bagherwal et al., 2018). Besides, our findings were also lower than a separate report on dogs (55.12%) conducted by Lakshmi et al., (2017). This variation in prevalence may be due to study on clinically suspected animals, difference in sample size and geographical variation. The figure in imaging techniques was lower and it may be due to morphological and parenchymal changes occurs only when the hepatic problem becomes more severe and in advance stage.

Though all ages are susceptible for liver disease in dogs and cats, older animals (more than 5 years old) were more susceptible (Bagherwal et al., 2018). Similarly, the hepatic problems were higher (50% both in dogs and cats) in old animals (more than 5 years old) than young (0% and 20% in dogs and cats respectively) and adults (0% and 5.17% in dogs and cats respectively) in our study. Similar observations were found by Maindigers et al., (2004) where reported highest hepatic problems were in older animals aged more than five years or more. This may be due to mechanical use of the liver and also reduced in body self immunity with progression of age.

In sex wise measurements, males (22.22% and 20.83% in dogs and cats respectively) were highly susceptible for hepatic problems than female (13.04% and 3.85% in dogs and cats respectively) in our study. This observation was opposed with the observations of Bagherwal et al., (2018) where reported females were more susceptible than male. But supported with the findings of Lakshmi et al., (2017) where they found males were more prone to hepatic problems. This difference may be due to disproportionate sampling in the study animals.

Breed wise predisposition revealed German shepherd (42.86%) was highest proportion followed by Lash Apsu (28.57%), Mixed breed (20.0%) and Local dog (12.50%). This finding was not agreement with study of Bagherwal et al., (2018) and Lakshmi et al., (2017) where they found higher proportion in Labrador Retriever followed by German Shepherd, Cross breed, Rottweiler etc. Higher proportion in German shepherd may be due to higher population of in the study area or disproportionate in sampling. In case of cat higher percentage of hepatic problems were found in local cat (19.05%) followed by Persian cat (10.53%). Although there is no breed predisposition for hepatic problems in

cats (Apalkova et al., 2013), higher percentage in local cat in this study may be due to increase tendency to visit outside that increase the chance of contact with other cats and dust.

V. CONCLUSION

Hepatic problems were highly found in clinical patients (dog and cat). Different breeds of male older aged animals were highly susceptible for hepatic problems in case of both dog and cat. Clinical history, clinical examination gave a clue of hepatic problems. Significantly altered hepatic enzymes made definitive diagnosis of hepatic problem as clinical signs become prominent in advance stage. Ultrasonography and x-ray were also helped to definitive diagnosis of hepatic problem in advance stage in some animals. According to our findings, it is highly suggestive to the pet owner to check their pets especially in older animal regularly to detect the hepatic problem in earlier and it will be helpful for effective treatment.

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Abbreviations and acronyms

ALP- Alkaline phosphatase
ALT- Alanine transaminase
AST- Aspartate aminotransferase
BUN- Blood urea nitrogen
CVASU- Chattogram Veterinary and Animal Sciences University

UGC- University grant commission

USG- Ultrasonography

P value- Pearson value

ml- mili liter

mg- mili gram

dl- deci liter

u/l- micro liter

%- percentage

Conflict of interest

There is no conflict of interest.

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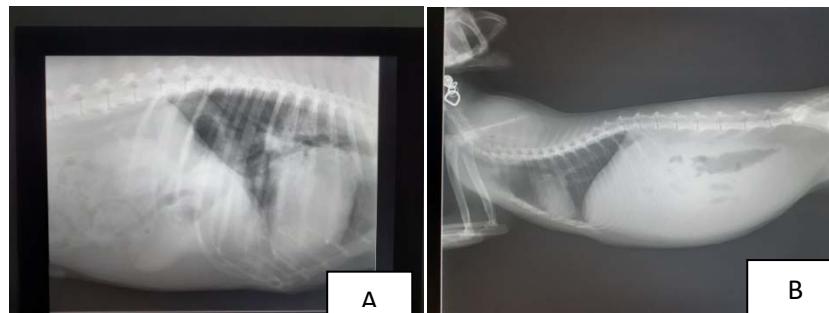


Figure 1: Hepatomegaly and borders are not sharpen (A); Fluid filled abdomen due to hepatic problem (B)

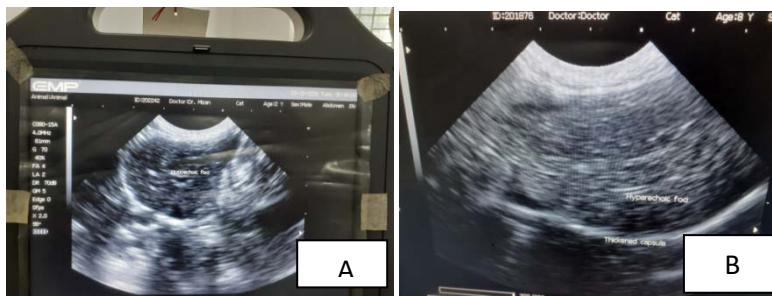


Figure 2: Hypoechoic foci on liver parenchyma (cyst or tumor) (A); Hyper echoic foci (necrosis or fibrosis tissue) and thickened liver capsule (B)

Table 1: Standard deviation and mean values of different biochemical parameters in dogs (n=50)

Parameters	Mean	Median	Standard deviation	Minimum	Maximum	Reference value
Bilirubin (mg/dl)	0.72±0.29	0.20	2.02	0.00	11.70	0.10-0.30
Total protein (mg/dl)	6.68±0.19	6.50	1.32	4.00	10.20	5.40-7.50
Albumin (mg/dl)	2.91±0.10	2.80	0.69	1.20	4.50	2.70-4.40
ALP (u/l)	333.86±127.63	61.35	902.48	10.70	5196.00	5.00-131.00
ALT (u/l)	256.36±88.62	67.45	626.66	18.50	3717.30	12.00-118.00
AST (u/l)	261.62±102.24	45.85	722.98	17.8	4154.40	15.00-66.00
Glucose (mg/dl)	84.92±2.96	84.60	20.97	37.5	130.80	70.00-138.00
Creatinine (mg/dl)	1.60±0.26	1.20	1.85	0.40	12.30	0.50-1.60
BUN (mg/dl)	29.46±3.90	20.17	27.58	10.30	154.40	6.00-25.00
Cholesterol (mg/dl)	132.52±6.71	120.00	47.43	59.00	280.60	92.00-324.00

Table 2: Standard deviation and mean values of different biochemical parameters in cats (n=50)

Parameters	Mean	Median	Standard deviation	Minimum	Maximum	Reference value
Bilirubin (mg/dl)	1.81±0.74	0.20	5.22	0.00	27.40	0.10-0.40
Total protein (mg/dl)	6.97±0.15	6.90	1.09	4.90	9.20	5.20-8.80
Albumin (mg/dl)	2.82±0.09	2.80	0.66	1.67	4.10	2.50-3.90
ALP (u/l)	245.42±79.79	34.60	564.22	7.80	2804.00	10.00-50.00
ALT (u/l)	180.16±42.43	45.65	300.06	12.30	1258.00	10.00-100.00
AST (u/l)	188.99±48.08	54.25	340.00	11.90	1704.40	10.00-100.00
Glucose (mg/dl)	98.22±4.95	87.40	35.02	45.60	232.60	50.00-170.00
Creatinine (mg/dl)	1.32±0.15	1.00	1.07	0.50	5.80	0.60-1.50
BUN (mg/dl)	31.52±2.56	27.10	18.12	12.60	110.40	14.00-36.00
Cholesterol (mg/dl)	119.26±6.10	104.00	43.11	67.00	218.60	75.00-220.00

Table 3: Frequency and percentage of different variables for hepatic problems in dogs

Explanatory variable	Co-variable	Total	+ve	Percentage	P value (χ^2 test)
Age	Adult	25	0	0	0.00
	Old	18	9	50	
	Young	7	0	0	
Sex	Female	23	3	13.04	0.40
	Male	27	6	22.22	
Breed	German shepherd	7	3	42.86	0.53
	Labrador	2	0	0.0	

	Lash apsu	7	2	28.57	
	Local	16	2	12.50	
	Mixed	10	2	20.0	
	Retriever	2	0	0.0	
	Rottweiler	2	0	0.0	
	Spitz	4	0	0.0	

Table 4: Frequency and percentage of different variables for hepatic problems in cats

Explanatory variable	Co-variable	Total	+ve	Percentage	P value (χ^2 test)
Age	Adult	39	2	5.13	0.00
	Old	6	3	50.0	
	Young	5	1	20.0	
Sex	Female	26	1	3.85	0.06
	Male	24	5	20.83	
Breed	Exotic	1	0	0.0	0.49
	Local	21	4	19.05	
	Mixed	9	0	0.0	
	Persian	19	2	10.53	

