

GERIATRIC CANINE MODEL FOR STUDY OF BIOCHEMICAL AND ULTRA-SONOGRAPHIC CHANGES IN AGE ASSOCIATED LIVER FAILURE

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ABSTRACT

In present study total number of 957 Dogs of different age group and different breeds presented to Referral Veterinary Polyclinic were used for study. Out of which 251 dogs were more than 5 years old and selected as geriatric dogs. Significantly ($P < 0.01$) lower levels of serum total protein (6.36 ± 1.31 mg/dl) albumin (1.68 ± 0.21 mg/dl) and calcium (7.14 ± 0.48 mg/dl) was found in geriatric dogs with liver disorders. There was significant increase in mean values of AST (244.95 ± 58.2 IU/L), ALT (280.77 ± 43.39 IU/L), total bilirubin (13.22 ± 2.14 mg/dl), Uric acid (3.83 ± 0.62 mg/dl) BUN (46.17 ± 3.36 mg/dl), GGT (23.78 ± 2.58 U/L) and NO (44.17 ± 13.87 μ mol/lit) in geriatric dogs with liver disorders. ultrasonographic examination of geriatric dogs of different breed, the most consistent finding was hepatomegaly, fatty liver, hepatitis, vacuolar and hyperechoic liver parenchyma. The present study can provide as model for understanding the disease mechanism in humans and possible amelioration.

1. INTRODUCTION

Aging is a universal phenomenon across all phyla of animal and plant kingdom. The causes of ageing include a variety of genetic determinants which embraces somatic and mitochondrial mutations, oxidative stress and protein glycation [1]. Data do not leave any doubt that geriatrics is naturally selected and genetically programmed in all species of animals [1]. The process of aging is influenced by many factors. The hypothalamic-pituitary-adrenal axis is an important hormonal system that governs homeostatic function. Current researches have disclosed that geriatrics is associated with increased cerebrospinal fluid and particularly

ventricular, cortisol levels and concomitant increased cortisol level in blood [2]. Aging results in organ failure and liver is supposed to be the prime organ affected by ageing process. The ageing process in geriatric population and its relation with hepatic function is a least studied process so far. It has been proposed that liver failure in adult dogs progresses through several stages. This effect is dependent on many factors such as nutrition, exercise, life style and quality of life provided to companion animal. Since all these factors are different from country to country, the effect of aging on organ function is also expected to be different than the developed countries. Dogs being closely associated with human have almost similar dietary habits and have to face similar environment as humans, so canines can be used as an appropriate model to study the diseases prevalent in human population. There is no systematic study on all these aspects in India. Keeping in view the above facts the present study was planned with objective of Screening of geriatric dogs suffering from liver failure for various metabolic and ultrasonographic changes and their comparison with healthy young dogs. The present study has significance for understanding the geriatric associated hepatic changes and can serve as important study for development of animal model to study liver failure in humans.

2. MATERIAL AND METHODS

2.1 STUDY ANIMALS

In present study total number of 957 Dogs of different age group and different breeds presented to Referral Veterinary Polyclinic were used for study. Out of which 251 dogs were more than 5 years old and selected as geriatric dogs. Dogs showing liver failure were categorized as diseased group and 30 normal healthy dogs presented for routine vaccination and deworming were categorized as healthy group for comparative study.

2.2 INCLUSION AND EXCLUSION CRITERIA

- i. Animals above five years of age (human equivalent) showing signs of liver failure were included in study.
- ii. Animals which have not undergone any medication which affects hepatic function were included while those dogs which have history of hepatotoxic medication were excluded from study.
- iii. Animals which have showed presence of other disease condition associated with liver failure were excluded from the study, with the sole purpose to include only those canines which were exclusively affected with hepatic failure.

2.3 SONOGRAPHIC EXAMINATION

The dogs were prepared for examination by clipping hair from the scanning site, cleaning of the skin with surgical spirit to remove debris and by application of liberal amounts of coupling gel to exclude air bubbles, which degrade image quality. Ultrasound images were obtained by using ultrasonographic equipment using either convex or linear array transducers of 5.0 to 7.5 MHz frequencies, depending on the size of the patient.

Sonographic images were printed using thermal printer. All abdominal ultrasonograms were recorded in dorsal recumbency. Ultrasonography was performed, as described [3], to evaluate abdominal organ echogenicity, to determine focal or diffuse abnormalities of the parenchyma.

2.4 SAMPLING

Blood (5.0 ml) was collected by venipuncture from the cephalic and/or radial vein using a disposable syringe after taking consent of the owners. Blood samples were centrifuged for 15 minutes at 3000 rpm to separate serum. Resulting sera were transferred to eppendorf micro tubes and frozen at -20°C till further use. This was used for estimation of various biochemical and endocrine parameters. Samples were taken from tumour for histopathology also.

2.5 BLOOD BIOCHEMISTRY

Creatine phosphokinase CPK was estimated as per the method of [4] and results were expressed as U/L. Alkaline phosphatase Alkaline phosphatase was estimated following the method of [5] and results were expressed as KA units. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzyme was measured following the method of [6] and Values were expressed as U/L. Serum total protein (g/dl) and albumin (g/dl) were determined by the modified Biuret and Dumas method [7]. Globulin was estimated by subtracting albumin from total protein. Urea concentration in serum (mg/dl) was measured by di-acetyl monoxime (DAM) Method [8] and urea nitrogen concentration was calculated by multiplying the urea concentration with factor 0.467 as indicated in the protocol of diagnostic kit. Serum Creatinine was estimated following alkaline picrate method [9]. Serum glucose concentration was determined following the method of [10] and results were expressed in mg/dl. Serum cholesterol was determined following the method of [11]. Serum Total bilirubin (mg/dl) was measured as per method described by [12]. Serum lactate dehydrogenase activity was determined by optimised DGKC, Kinetic assay method [13]. The values of LDH were expressed in IU/L. Phosphorus Phosphorus concentration in serum was estimated as per [14] UV Molybdate end point assay method. The values of phosphorus were expressed in mg/dl. Calcium in serum was estimated as per Miller, (1994) UV Molybdate end point assay method. The values of phosphorus were expressed in mg/dl.

2.6 STATISTICAL ANALYSIS

Data was analyzed statistically using SPSS software, Statistical Analysis. Data were subjected to statistical analysis

3 RESULTS

Out of 251 geriatric dogs 35 (13.95%) had clinical evidence of liver diseases. Dogs showed clinical signs of liver disease viz jaundices (Fig. 1 and 2) ascites (Fig. 3). Serum biochemical changes in these dogs are presented

in (Table 1). Significantly ($P < 0.01$) lower levels of serum total protein (6.36 ± 1.31 mg/dl) albumin (1.68 ± 0.21 mg/dl) and calcium (7.14 ± 0.48 mg/dl) was found in geriatric dogs with liver disorders. There was significant increase in mean values of AST (244.95 ± 58.2 IU/L), ALT (280.77 ± 43.39 IU/L), total bilirubin (13.22 ± 2.14 mg/dl), Uric acid (3.83 ± 0.62 mg/dl) BUN (46.17 ± 3.36 mg/dl), GGT (23.78 ± 2.58 U/L) and NO (44.17 ± 13.87 μ mol/lit) in geriatric dogs with liver disorders.

In the present study, ultrasonographic examination of geriatric dogs of different breed, the most consistent finding was hepatomegaly, fatty liver, hepatitis, vacuolar and hyperechoic liver parenchyma. Dogs with hepatomegaly and hepatitis had ascites, increased bilirubin and ALT level. Normally, the liver should be slightly more echogenic or isoechoic (same echogenicity) when compared with the renal cortex; and should be hypoechoic compared with the splenic parenchyma. The ecostructure of liver and gall bladder revealed thickened wall of gall-bladder Cholecystitis (Fig. 4), Hepatomagaly (Fig. 5) and Hepatitis (Fig.6).

4. DISCUSSION

In our study ALT was significantly ($P < 0.01$) higher in dogs of various breeds with liver diseases. Increased ALT activity levels in serum are used to detect liver damage [15]. Alanine aminotransferase is a hepatocyte-specific enzyme. An increase in its serum activity is principally due to hepatic parenchymal disease. In this study the simultaneous increase in ALT and AST activity suggests a hepatic disorder. In liver injury, ALT and other enzymes leak out from necrotic cells. We found that other liver injury markers (AST, GGT, albumin, TP, bilirubin and ALP) were also higher in geriatric dog with liver failure. In our study GGT was significantly ($P < 0.01$) higher in geriatric dogs with liver diseases. GGT is generally known to be elevated in most subjects with liver disease regardless of cause. Alkaline phosphatase is thought to be useful in evaluating biliary disease in dogs. Hepatobiliary or bone disease, hyperadrenocorticism, and glucocorticoid or anticonvulsant administration can cause increased ALP values [16]. In the exocrine pancreatic insufficiency dogs, since in our study other causes were excluded, hepatopathy was thought to be the main reason for the increased ALT, AST, and ALP activity. Bilirubin concentrations were found higher in geriatric dogs with liver failure. However, there must be considerable hepatocellular disease for hyperbilirubinemia to occur, because the liver reserve capacity for bilirubin processing is up to 30 times the normal bilirubin load [17].

Ultrasound imaging has many advantages, namely its low cost, extensive availability and the fact that it can be easily done in canine model. Moreover, it is an accurate, innocuous and non-invasive technique and it is the most commonly used method for the investigation of liver diseases in humans and small animals [18]. Healthy dogs showed homogeneous liver parenchyma, with medium level echogenicity and regular liver surface, as previously described in humans [19] and cats [20,21]. Fatty liver disease in dogs were characterized by homogeneous and diffusely increased echogenicity ("bright liver"), equal or greater to the right renal cortex, reduced visualization of the diaphragm and of small peripheral vessels, and by no changes in liver surface, as described in humans [22] and cats [23]. The presence of ascites, which is also an important indicator of the

severity of liver disease, was detected even when the amount of fluid present in the peritoneal cavity was small, as previously reported by [21].

5. CONCLUSION







So it can be concluded that with age there is decreased liver function as indicated by altered liver function and elevated liver specific enzymes. Similarly on sonographic examination most of the features observed in geriatric liver failure were similar to those observed earlier in human liver failure. The present study will be helpful to understand the pathogenesis of age associated hepatic failure in other economically important animal species and human and development of effective therapeutic and prophylactic protocol against liver failure.

Table 1: Biochemical changes in geriatric dogs with liver disorder

Parameters	Group 1 (Healthy control)	Group 2 (Liver disorder)
Total protein (g/dl)	8.02±1.52	6.36±1.31**
Albumin (g/dl)	4.33±1.31	1.68±0.21**
Globulin (g/dl)	3.10±1.27	4.63±1.39
Glucose (mg/dl)	101.31±9.28	81.63±9.26*
Cholesterol (mg/dl)	145.36±17.85	221.04±10.53**
Total bilirubin (mg/dl)	9.63±1.80	13.22±2.14
Creatinine (mg/dl)	1.10±0.24	1.82±0.13*
Uric acid (mg/dl)	0.760±0.091	3.83±0.62**
BUN (mg/dl)	28.45±2.25	46.17±3.36**
GGT (U/L)	5.58±0.75	23.78±2.58**
ALP (U/L)	35.68±5.53	49.68±5.27*
ALT U/L)	126.22±18.36	380.77±43.59**
AST U/L)	99.31±18.27	244.95±58.27**
NO (µmol/lit)	9.36 ±1.269	44.17±13.87**

Values with superscript * differ significantly (P<0.05) between the group.

Values with superscript ** differ significantly (P<0.01) between the group

		
Fig.1: Icteric mucous membrane	Fig.2: Icteric conjunctiva	Fig.3: ascitis
		
Fig.4: Hepatomegaly	Fig.5: Liver lobes floating in ascitic fluid	Fig.6: Cholecystitis accompanying hepatomegaly.

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