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Role of blood beta-hydroxybutyric acid estimation as a diagnostic marker of feline hepatic lipidosis#

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Abstract

Feline hepatic lipidosis (FHL), characterised by an accumulation of triglycerides in the cytoplasm of hepatocytes, is a common and potentially fatal liver disorder in cats. Hepatic lipidosis in cats can develop due to any condition that will impair nutrient uptake and is often presented with non-specific clinical signs. The present study describes the diagnosis of FHL based on clinico-biochemical, ultrasonographic, and cytological changes and evaluates the diagnostic utility of blood beta-hydroxybutyric acid (βHBA) estimation in FHL. Anorexia, weight loss, lethargy, vomiting, dehydration, and jaundice were the common clinical findings in cats with hepatic lipidosis. Serum biochemical evaluation revealed elevations in alkaline phosphatase (ALP), triglycerides, glucose, and total bilirubin. Ultrasonography revealed an enlarged hyperechoic liver. Fine-needle aspiration cytology of the liver revealed mild to severe vacuolation in the cytoplasm of hepatocytes. Blood beta-hydroxybutyric acid levels were found higher in cats with hepatic lipidosis than in healthy cats and cats with other hepatic disorders. Therefore, βHBA estimation, being a quick and non-invasive method, could be considered as a diagnostic marker in the early diagnosis of FHL.

Keywords: *Hepatic lipidosis, beta-hydroxybutyric acid (βHBA), liver*

The most common hepatobiliary disease in cats is hepatic lipidosis (Webb, 2018). It can

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be primarily due to decreased food intake or secondary as a consequence of an underlying disease. Feline hepatic lipidosis (FHL) needs to be rapidly diagnosed and corrected, as it is an acute critical syndrome that can result in the animal's death due to severe liver dysfunction and failure if therapeutic measures are not taken expeditiously (Valtolina and Favier, 2017).

The diagnosis of FHL is based on clinical findings, haemato-biochemical evaluation, diagnostic imaging, and cytological examination of the liver. Ketones (beta-hydroxybutyric acid (βHBA), acetoacetate (AA), and acetone) in the body reflect utilization of fat as an energy source where energy needs exceed the intake and thus reflect states of negative energy balance (NEB) (Gorman *et al.,* 2016). Urine dipstick analysis detects only AA and acetone and does not detect blood βHBA. Serum βHBA had better sensitivity to determine NEB than dipstick examination of urine for ketones because hyperketonaemia occurred more frequently than ketonuria during NEB (Aroch *et al.,* 2012). Since NEB predisposed cats to FHL irrespective of the aetiology, serum βHBA measurement has the potential to be used as a marker of caloric stress. Thus, the present study aimed to evaluate the diagnostic utility of blood βHBA estimation in the diagnosis of FHL.

Materials and methods

Twenty domestic cats, irrespective of age, sex, and breed presented to TVCC, CVAS, Pookode, Wayanad, suspected of hepatobiliary disorders were selected for the study. Six apparently healthy cats brought for routine health check-ups were taken as the control group for comparison of the parameters under study.

The haematological evaluation was done using a three-part fully automated haematological analyzer (Mindray BC-2800Vet), and biochemical parameters such as serum ALT, ALP, GGT, total protein, albumin, globulin, total bilirubin, glucose, total cholesterol, and triglycerides were estimated using diagnostic kits in a semi-automatic biochemical analyzer (Agappe MISPAVIVA 2578-10/17). A peripheral blood smear examination was performed to observe any abnormalities in the morphology of RBC and to rule out haemoprotozoans. Blood glucose (mg/dL) and βHBA levels (mmol/L) were estimated using the FreeStyle Libre reader with the specific test strips for glucose and ketones. Ultrasonographic scanning of the liver was performed in dorsal, left or right lateral recumbency using high-frequency linear sonographic probes (≥ 7.5 MHz). The fine needle aspiration cytology (FNAC) specimens were obtained by non-aspiration technique under ultrasound guidance, where a 24-gauge needle with a syringe already filled with 5ml of air attached was inserted into the liver. The needle and syringe were then rapidly moved back and forth in the tissue and the aspirated material was then ejected onto the slide, and smears were prepared immediately. The non-aspiration technique of collecting the fine needle aspirates was preferred for the sampling of the liver to minimise blood contamination (Dunn, 2014).

Statistical analysis was done using SPSS software version 24.0 and applied independent t-test (Snedecor and Cochran, 1994).

Results and discussion

In the present study, hepatic lipidosis accounted for the highest incidence (40 per cent; 8 out of 20 cats) among the hepatobiliary conditions affecting the cats. Kuzi *et al.* (2017) documented that hepatic lipidosis affects about 50 per cent of cats presented with liver disease. In the present study, the most common breed affected was the Persian and most cats were younger than 3 years, weighing above 2 kg. Both male and female cats were affected equally. Common presenting complaints were anorexia, weight loss, lethargy, vomiting, dehydration, and jaundice (Fig 1).

The mean ± SE values of haematological findings between the control and study groups are given in Table 1. There was no statistically significant difference in haematological parameters between cats with hepatic lipidosis and healthy control cats. Valtolina and Favier (2017) observed a normal complete blood count results in cats with FHL.

The mean ± SE values of serum biochemical parameters of cats affected with hepatic lipidosis are shown in Table 2. There was a significant increase in ALP ($p<0.05$), triglycerides ($p<0.01$), and total bilirubin ($p<0.01$) in the hepatic lipidosis group than the control group. Increased serum activities of ALP, ALT, and total bilirubin in cats were observed by Center (2005) in hepatic lipidosis (HL) in cats. Brown *et al*. (2000) observed that hypertriglyceridaemia, rather than hypercholesterolaemia, was more frequently found in patients with HL. A significant decrease in the A: G ratio ($p<0.01$) and a mild decrease in albumin was observed in cats with HL in the present study. Mild hypoalbuminemia was reported in feline HL secondary to anorexia and decreased hepatic function (Valtolina and Favier, 2017). Intrahepatic cholestasis, which might result from hepatocyte enlargement brought on by hepatic lipidosis was the aetiology of hyperbilirubinemia (Weingarten and Sande, 2015) and increased ALP levels.

On peripheral blood smear examination, abnormally shaped erythrocytes (poikilocytes) were observed in some cases (Fig 2). Poikilocytes and Heinz bodies were found in cats with hepatic lipidosis, though those were non-specific findings (Armstrong and Blanchard, 2009; Center, 2005). RBC morphology may be altered due to changes in plasma cholesterol and lipid levels and the resulting abnormally shaped erythrocytes may have shortened survival times making it prone to fragmentation (Rutgers, 1998).

There was a significant increase in blood glucose level ($p<0.01$) in the hepatic lipidosis group than the control group. Hyperglycaemia was present in FHL as a result of stress, diabetes mellitus, or acute severe pancreatitis (Webb, 2018).

Blood beta-hydroxybutyric acid levels were estimated using the FreeStyle Libre reader with the specific ketone strips (Fig 3). There was a significant increase in βHBA level ($p<0.05$) hepatic lipidosis group to the control group and cats with other hepatic disorders (Table 3). Blanchard *et al*. (2004), Aroch *et al.* (2012), Cooper *et al.* (2015) and Gorman *et al.* (2016) observed an increase in βHBA levels in cats with hepatic lipidosis. Urine dipstick analysis detects only acetoacetate (AA) and acetone and does not detect blood βHBA, which is often considerably higher than urinary AA and hyperketonaemia occurs more frequently than ketonuria in negative energy balance (Laffel, 1999; Aroch *et al.,* 2012). Measurement of serum βHBA therefore has a higher sensitivity than dipstick analysis of urinary ketones and thus is a good predictor of FHL.

On ultrasonographical examination, more than 60 per cent of cats showed an enlarged liver and hyperechoic parenchyma (Fig 4). Hyperechogenicity of the liver relative to the falciform fat has been reported to be the best criterion for diagnosis, with a sensitivity of 91 per cent for the detection of severe FHL (Yeager and Mohammed, 1992).

The cytological examination of the liver using fine needle aspiration samples was done to confirm the condition. In the present study, among the cats affected with hepatic lipidosis, 25 per cent of cats had a mild degree of lipidotic changes with less than 20 per cent of hepatocytes with small vacuoles, 37.5 per cent of cats had a moderate degree of lipidotic changes with nearly 20-80 per cent of hepatocytes affected with small, medium, or large vacuoles. In cats with severe hepatic lipidosis, more than 80 per cent of hepatocytes were affected and the cytoplasm of the hepatocytes was markedly distended by large, clear vacuoles (Table 4, Fig 5-8). Feline hepatic lipidosis was characterised by lipid accumulation in the hepatocytes and the cytoplasm of the hepatocytes was markedly distended by large, clear vacuoles (macrovesicular or microvesicular) that caused nuclear margination or a “signet ring” appearance and made the hepatocyte difficult to recognize (Wang *et al*., 2004; Dunn, 2014; Raskin and Meyer, 2010).

All the cats diagnosed with HL were completely anorectic for more than 3 days. The primary reasons for the development of HL in cats in the present study included changes in the diet and environment. Hepatic lipidosis was also observed secondary to other infections like feline panleukopenia in the present study.

Table 1. Comparison of day 0 haematological parameters between control and hepatic lipidosis in cats

Parameters	Control	Hepatic lipidosis (n=8)	F-value	P-value
TEC (x 10^6 cells/mm^3)	8.23 ± 0.69	8.78 ± 0.84	1.367^{ns}	0.279
Hb (g/dL)	12.18 ± 0.96	10.11 ± 1.20	2.863^{ns}	0.06
VPRC (%)	36.24 ± 2.86	38.78 ± 4.50	4.105^{ns}	0.019
TLC (x 10^3 cells/mm^3)	11.7 ± 1.24	11.63 ± 2.76	5.304^{ns}	0.007
Platelets (x 10^3 cells/mm^3)	316.33 ± 54.31	302.50 ± 58.79	2.406^{ns}	0.095
Neutrophils (%)	51.57 ± 3.97	56.45 ± 5.83	6.756^{ns}	0.002
Lymphocytes (%)	38.05 ± 3	29.55 ± 5.01	4.227^{ns}	0.017
Monocytes (%)	3.22 ± 0.25	4.70 ± 0.69	1.812^{ns}	0.174

*** Significant at 0.01 level; * Significant at 0.05 level; ns nonsignificant*
Means having different letters as superscripts differ significantly

Table 2. Comparison of day 0 biochemical findings between control and hepatic lipidosis in cats

Parameters	Control	Hepatic lipidosis (n=8)	F-value	P-value
ALT (IU/L)	36.45 ± 8.2	174.90 ± 36.07	11.965	<0.001
ALP (IU/L)	20.18 ± 4.59	148.98 ± 40.40	3.813*	0.024
GGT (IU/L)	1.09 ± 0.38	3.29 ± 0.91	10.472	<0.001
Total protein (g/dL)	6.69 ± 0.14	6.23 ± 0.42	1.623^{ns}	0.213
Albumin (g/dL)	2.81 ± 0.16	2.17 ± 0.24	1.357^{ns}	0.282
Globulin (g/dL)	3.88 ± 0.16	4.34 ± 0.33	4.045	0.02
A: G ratio	0.73 ± 0.061	0.478 ± 0.050	11.489**	<0.001
Total cholesterol (mg/dL)	107.69 ± 9.54	121.59 ± 27.90	1.838^{ns}	0.17
Triglycerides (mg/dL)	65.93 ± 9.16	276.73 ± 64.62	7.135**	0.002
Total bilirubin (mg/dL)	0.133 ± 0.033	5.903 ± 1.276	11.47**	<0.001

*** Significant at 0.01 level; * Significant at 0.05 level; ns non significant*
Means having different letters as superscripts differ significantly

Table 3. Comparison of day 0 blood glucose and ketones between control and hepatic lipidosis in cats

Parameters	Control	Hepatic lipidosis (n=8)	F-value	(P-value)
Glucose (mg/dL)	103.33 ± 6.83	177.50 ± 19.42	11.607**	<0.001
βHBA (mmol/L)	0.067 ± 0.033	2.638 ± 1.010	4.264*	0.016

*** Significant at 0.01 level; * Significant at 0.05 level; ns non significant*
Means having different letters as superscripts differ significantly

Table 4. Classification of the degree of feline hepatic lipidosis by cytological examination

Degree	Size of vacuoles	Affected hepatocytes	Number of cases (n=8)	Percentage
Mild	Small	< 20 %	2	25
Moderate	Small and medium	20-80 %	3	37.5
Severe	Small, medium and large	> 80 %	3	37.5

Fig. 1: Clinical signs
A: Weakness and poor body condition

B: Icteric mucous membrane

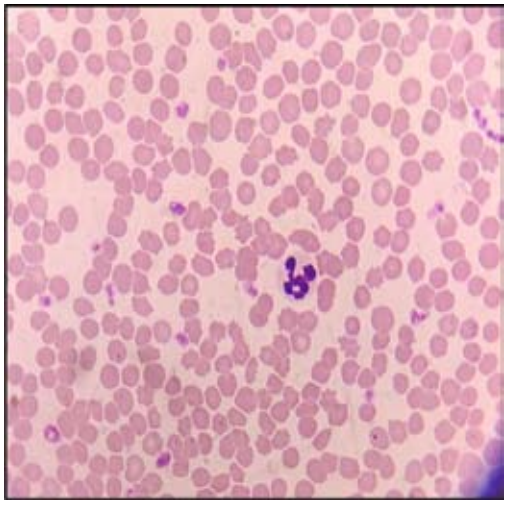

Fig. 2: Poikilocytosis (abnormal shaped RBCs including acanthocytes) in blood smear evaluation.

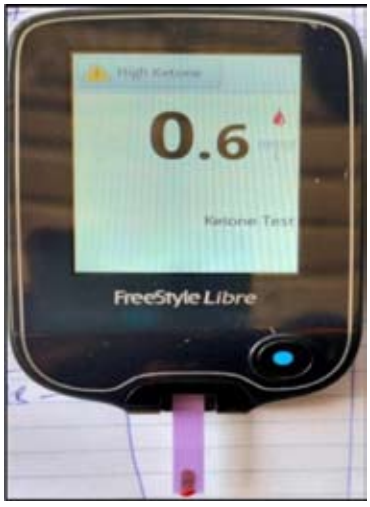

Fig. 3: FreeStyle Libre reader with optium H β- ketone test strip (Abbott Diabetes Care Ltd).

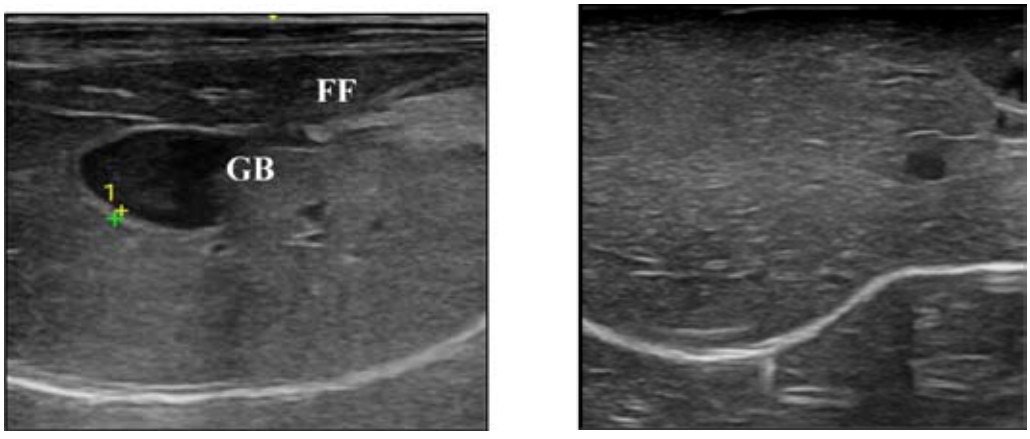

Fig. 4: Ultrasonography of the liver
Increased echogenicity (hyperechoic to falciform fat) in a cat with hepatic lipidosis. GB- gall bladder, FF- falciform fat.

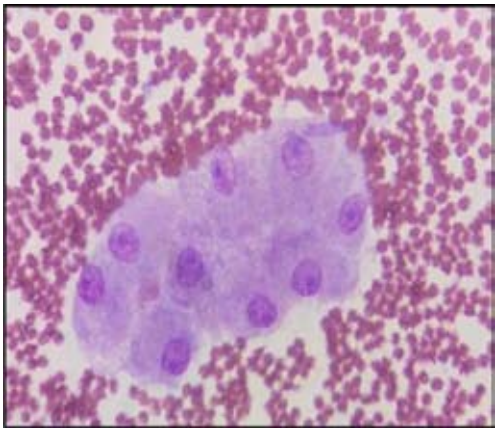

Fig. 5: Cytology of the liver. A cluster of feline normal hepatocytes (Leishman stain, 1000X).

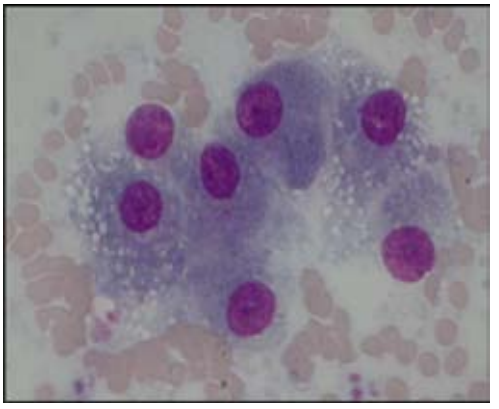

Fig. 6: Few hepatocytes with microvescicles indicating a mild degree of hepatic lipidosis in a cat (Leishman stain, 1000X).

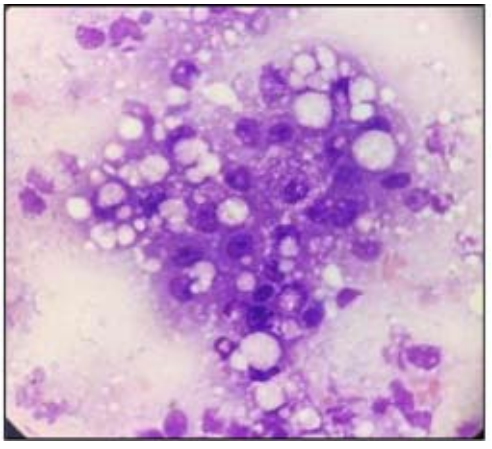

Fig. 7: Hepatocytes with both small and large vacuoles indicating a moderate degree of hepatic lipidosis in a cat (Leishman stain, 1000X).

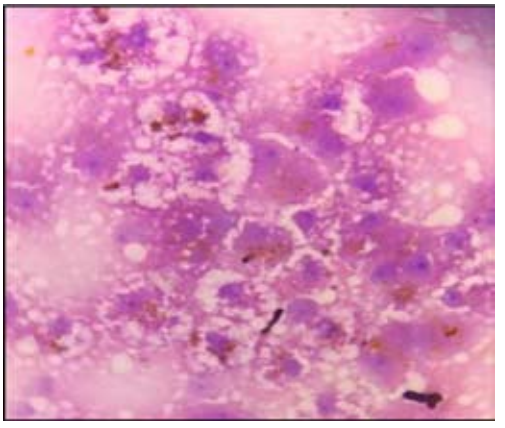

Fig. 8: Marked cytoplasmic vacuolization and nuclear pyknosis with all hepatocytes indicating a severe degree of hepatic lipidosis in cats (Leishman stain, 1000X).

Conclusion

Blood beta-hydroxybutyric acid levels were found higher in cats diagnosed with hepatic lipidosis. Being a quick and non-invasive method, βHBA estimation could be considered as a diagnostic marker of FHL as it was found to be a good predictor of this condition so that appropriate treatments could be instituted quickly.

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Conflict of interest

The authors declare no conflict of interest.

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