A descriptive study of the histopathologic and biochemical liver test abnormalities in dogs with liver disease in Thailand

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Abstract

The present study describes the serum biochemical alterations and histopathological abnormalities in the liver tissue of dogs with liver disease. A survey of hepatic lesions was conducted using ultrasound-guided percutaneous needle biopsies. The hematologic and biochemical changes in dogs with liver lesions were recorded. Chronic hepatitis was the most common liver histopathologic finding (37.9%). Other common findings included liver fibrosis (19.5%), vacuolar hepatopathy (10.3%), cholangiohepatitis (9.2%), hepatocellular carcinoma (4.6%), cholangitis (3.4%), cholangiocarcinoma (2.3%), and congestive hepatopathy (2.3%). Greater than 2-fold elevation of serum alanine aminotransferase (ALT) was a useful indicator, with a sensitivity of 40% to 65% for diagnosing all liver pathologies. Greater than 2-fold elevation of serum alkaline phosphatase (ALP) without significant elevation of ALT was useful for diagnosing liver diseases affected by inflammatory or regressive changes (sensitivity of 40% to 50%). Elevation of ALT, ALP, or a combination of ALT and ALP had a high sensitivity of up to 90% for identifying dogs with liver pathology. Hepatic injury and cholestasis enzymes should be interpreted together with patient history, clinical signs, and liver ultrasonographic appearance before performing a liver biopsy.

Résumé

La présente étude vise à décrire les modifications biochimiques sériques et les anomalies histopathologiques dans le tissu hépatique de chiens avec des maladies du foie. Une analyse des lésions hépatiques fut menée en utilisant des biopsies à l'aiguille percutanées guidées par échographie. Les changements hématologiques et biochimiques chez les chiens avec des lésions hépatiques furent notés. L'hépatite chronique était la trouvaille histopathologique du foie la plus fréquente (37,9 %). D'autres trouvailles fréquentes incluaient la fibrose du foie (19,5 %), l'hépatopathie vacuolaire (10,3 %), la cholangio-hépatite (9,2 %), le carcinome hépatocellulaire (4,6 %), la cholangite (3,4 %), le cholangiocarcinome (2,3 %) et l'hépatopathie congestive (2,3 %). Une augmentation de plus du double de l'alanine aminotransférase sérique (ALT) était un indicateur utile, avec une sensibilité de 40 % à 65 % pour diagnostiquer toutes les pathologies du foie. Une augmentation de plus du double de la phosphatase alcaline sérique (ALP) sans augmentation significative d'ALT était utile pour diagnostiquer des maladies hépatiques avec des changements inflammatoires ou régressifs (sensibilité de 40 % à 50 %). Une augmentation d'ALT, d'ALP ou une combinaison d'ALT et d'ALP avait une sensibilité élevée jusqu'à 90 % à identifier les chiens avec une pathologie du foie. Les blessures hépatiques et les enzymes d'une cholestase devraient être interprétés conjointement avec l'histoire du patient, les signes cliniques et l'apparence échographique du foie avant d'effectuer une biopsie du foie.

(Traduit par Docteur Serge Messier)

Introduction

Hepatobiliary diseases are one of the most important causes of morbidity and mortality in dogs and cats (1). In contrast to humans, histopathologic examination is required to diagnose most canine liver diseases, especially parenchymal liver diseases (2). The histological criteria for diagnosing liver diseases in dogs have been standardized by the World Small Animal Veterinary Association (WSAVA). Liver biopsy techniques for histology include percutaneous ultrasound-guided needle biopsy, laparoscopic cup forceps, and surgical biopsy during laparotomy (3). The usage of a percutaneous ultrasound-guided 14-gauge needle biopsy in dogs to obtain samples that contain at least 3 to 12 portal triads is suggested for accurate histologic diagnosis of liver diseases (4,5). The specimens of the liver from an 18-gauge needle biopsy are often small and should be interpreted with caution (3,6). Increased alanine aminotransferase (ALT) serum activity in canines indicates damage of the hepatocellular membrane and is often the earliest indicator of canine liver diseases (7,8). If ALT levels are greater than twice the upper limit of the reference interval for more than 4 wk, further investigation of canine liver diseases using histopathology is needed (3). However, the overall sensitivity of ALT for canine liver disorders is about 60%, which could be as low as 40% in neoplasia and as high as 100% in hepatic necrosis (7). Diseases involving a reduced number of hepatocytes, such as cirrhosis, may be present even in the absence of elevated ALT (8).

Serum alkaline phosphatase (ALP) is released in cholestatic liver diseases. The sensitivity of ALP for detecting parenchymal liver disorders varies between 40% and 90% (7). It has been suggested that increased ALP activity occurs later in the course of diseases such as

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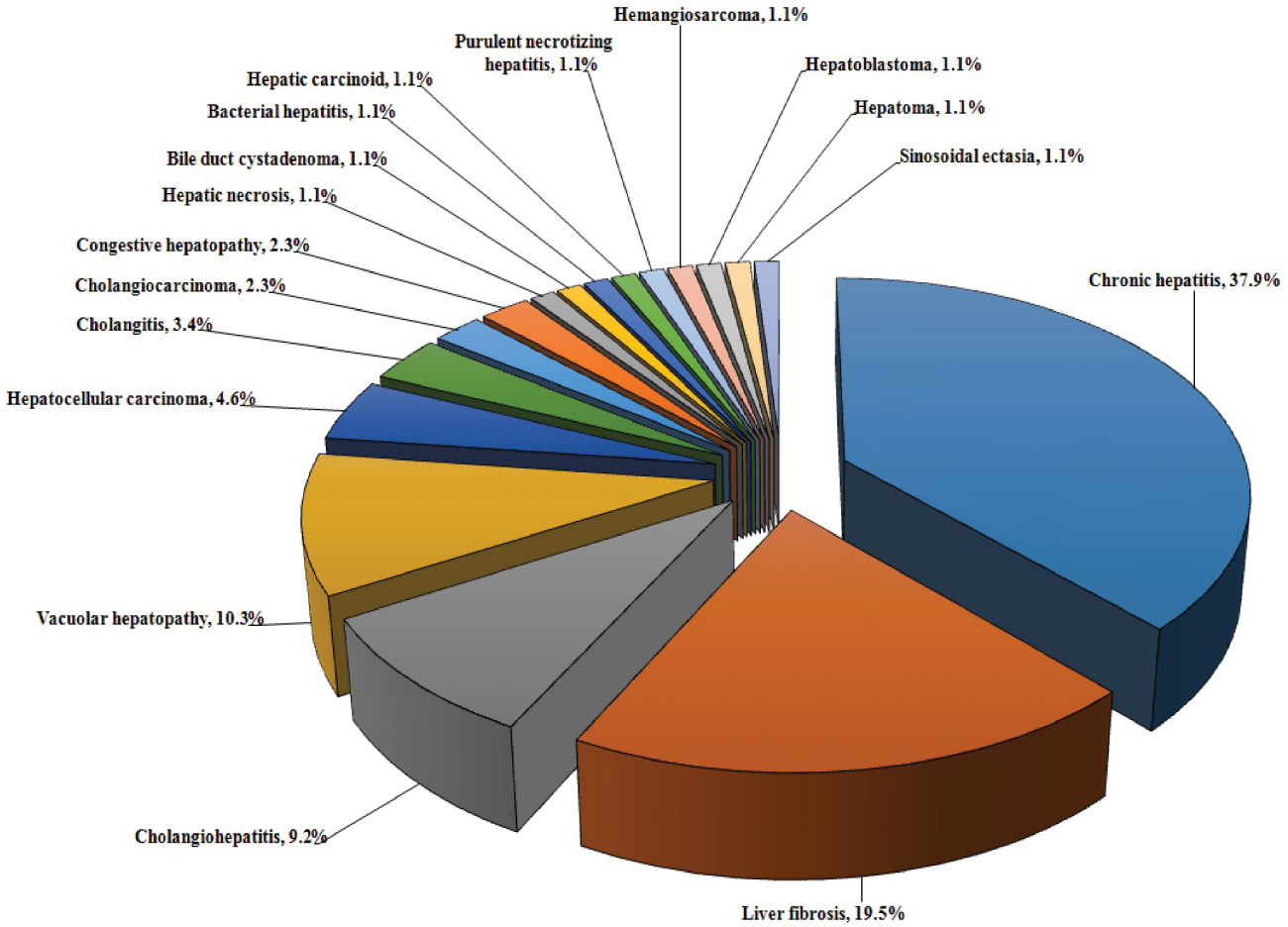

Figure 1. Distribution of histopathologic diagnoses from canine liver samples obtained using an ultrasound-guided Tru-Cut biopsy.

chronic hepatitis (9). Up to 20% of dogs with chronic hepatitis can present with increased serum liver enzyme activity in the absence of clinical illness (9–11).

Another indicator for conducting a liver biopsy is ultrasound imaging abnormalities, such as masses or diffuse echotextural changes. A definitive diagnosis of canine hepatic disease cannot be made from ultrasonographic appearance. Previous studies have found that no relationship exists between any of the ultrasonographic criteria and microscopic diagnoses or laboratory values (12–14). A recent study demonstrated that hepatobiliary ultrasound score may have potential as a screening tool to detect some groups of liver diseases (15). Thus, clinical signs, laboratory data, and imaging results should be considered before conducting a liver biopsy.

Previous research has suggested that hepatitis is a common histologic finding in dogs (11,16–19). Retrospective studies of histological diagnoses of canine hepatic diseases have been reported in the United States and Japan (14,20). However, the histopathologic features of canine hepatic diseases in Thailand have never been described. Thus, a study of the prevalence of canine liver diseases would be useful to help guide veterinarians to perform appropriate diagnostic tests.

The purpose of this study was to describe different types of canine hepatic diseases in Bangkok, Thailand, including clinical, laboratory, and pathologic findings. We also determined the sensitivity of certain hepatocellular and cholestasis markers of liver diseases for detecting histopathologic changes.

Materials and methods

The use of animals in this study was approved by the Kasetsart University Institutional Animal Care and Use Committee (ACKU61-VET-012). Included in the study were canine patients with i) persistent (more than 3 mo) unexplained elevated liver enzymes (ALT and/or ALP) of greater than twice the upper limit of the reference range, or ii) less than twice the upper limit of the elevated liver enzymes but with abnormal ultrasonographic liver parenchymal appearance (i.e., irregular hypoechoic nodules, heterogenous liver echotexture). Patients with elevated liver enzymes due to extrahepatic diseases such as hyperadrenocorticism, exposure to chronic hepatotoxic drugs, or evidence of known systemic infectious disease were excluded. A total of 87 dogs were evaluated with each owner's informed consent during January 2016 to January 2018. Dogs with a body weight of 0 to 12 kg, 12.01 to 24 kg, and $>$ 24.01 kg were described as small, medium, and large breeds, respectively.

Liver biopsies were obtained from animals under general anesthesia using 14-gauge automatic spring-loaded needles (Argon, Frisco, Texas, USA) with ultrasound (GE, Chicago, Illinois, USA) guidance.

Table I. Alteration of hematology and biochemical parameters in dogs with a liver lesion.

Variables	Number of dogs	Median	Minimum	Maximuim	Cut-off value	Number of dogs with abnormal value (%)	Number of dogs with normal value (%)
PCV (%)	87	41.7	23.8	57.4	< 35	14 (16.1)	73 (83.9)
WBC ($\times 10^3$/cumm)	87	10 100	2500	39 000	> 17 000	8 (9.2)	79 (90.8)
Platelets ($\times 10^3/\mu$L)	87	299.5	8.0	1068	< 200	16 (18.4)	71 (81.6)
BUN (mg%)	87	14.5	4.0	57	< 10	13 (14.9)	74 (85.1)
Creatinine (mg%)	87	0.8	0.4	2.4	> 1.3	4 (4.6)	83 (95.4)
ALT (IU/L)	87	137	22	4797	> 140	42 (48.3)	45 (51.7)
AST (IU/L)	86	34	14	847	> 86	11 (12.8)	75 (87.2)
ALP (IU/L)	87	428	33	11 335	> 152	67 (77.0)	20 (23.0)
GGT (IU/L)	85	5	0	296	> 4	47 (55.3)	38 (44.7)
Bile acid (μM/L)	83	9	5	30	> 12	28 (33.7)	55 (66.3)
Bilirubin (mg%)	86	0.1	0	15.3	> 0.5	9 (10.5)	77 (89.5)
Albumin (mg%)	87	3.3	1.5	4.6	< 2.3	4 (4.6)	83 (95.4)
Globulin (mg%)	87	3.6	2.6	9.1	> 3.9	26 (30.0)	61 (70.0)
PT (s)	86	5.5	2.4	8.9	> 7.5	2 (2.3)	85 (97.7)
aPTT (s)	86	14.1	9.9	20	> 18.4	2 (2.3)	85 (97.7)

PCV — packed cell volume; WBC — white blood cell; BUN — blood urea nitrogen; ALT — alanine aminotransferase; AST — aspartate aminotransferase; ALP — alkaline phosphatase; GGT — gamma-glutamyl transferase; PT — prothrombin time; aPTT — activated partial thromboplastin time.

At least 3 pieces of tissue were collected from more than one liver lobe to accurately diagnose liver disease (21). A Thai Board-certified pathologist performed the histology of those collected liver samples. Most diagnoses were based on the WSAVA criteria (2), but we also adopted other diagnoses that have not yet been classified by the WSAVA criteria. The histological diagnoses of canine liver diseases in this study were grouped into the following categories according to Hirose et al (20): inflammatory diseases (i.e., chronic hepatitis, cholangitis, cholangiohepatitis, bacterial hepatitis, purulent necrotizing hepatitis, liver fibrosis), neoplasia (i.e., hepatocellular carcinoma, hepatoma, hepatoblastoma, cholangiocarcinoma, bile duct cystadenoma, hepatic carcinoid, hemangiosarcoma), regressive change (i.e., vacuolar hepatopathy, hepatic necrosis), and circulatory disturbance (i.e., congestive hepatopathy, sinusoidal ectasia).

Blood samples were collected on the same day as the liver biopsy from all dogs *via* a cephalic or saphenous vein to determine complete blood (cell) count (CBC) and blood chemistry, including blood urea nitrogen, creatinine, ALT, aspartate aminotransferase (AST), ALP, gamma-glutamyl transferase (GGT), preprandial bile acid, total bilirubin, total protein, albumin, prothrombin time (PT), and activated partial thromboplastin time (aPTT).

The data were analyzed using GraphPad Prism Version 6 (GraphPad Software, San Diego, California, USA). All continuous variables were analyzed for normal distribution after logarithmic transformation. The continuous variables were tested using Student's *t*-test and the X^2-test for categorical variables. Data were expressed as mean ± standard deviation. A P-value < 0.05 was considered statistically significant. Pearson's correlation test *(r)* was applied to determine the relationship between liver enzymes (ALT, AST, ALP, and GGT) and the relationship between liver enzymes and liver function tests (bile acid, albumin, bilirubin, PT, and aPTT)

Results

Of the 87 dogs included in this study, 39 (44.8%) were male and 48 (55.2%) were female. The average age of the dogs at the time of presentation was 13.4 y and the median age was 10.6 y (range: 1.7 to 16.5 y). Twenty breeds were represented and small, medium, and large breeds accounted for 49 (57.3%), 34 (39.1%), and 4 (4.6%) dogs, respectively. Mixed-breed dogs represented the largest proportion of the sample at 26.4%. Other breeds represented included shih tzu (16; 18.4%), poodle (10; 11.5%), Chihuahua (7; 8.1%), Siberian husky (5; 5.8%), West Highland white terrier (4; 4.6%), beagle (4; 4.6%), Labrador retriever (3; 3.5%), miniature schnauzer (3; 3.5%), Maltese (2; 2.3%), and golden retriever (2; 2.3%). In addition, the sample included 1 (1.2%) of each of the following breeds: English cocker spaniel, American pit bull terrier, Thai Bangkaew, French bulldog, Jack Russell terrier, miniature pinscher, Pomeranian, and smooth fox terrier. Most dogs (73; 83.9%) did not display abnormal clinical signs. Only 5 (5.7%) had decreased appetite or anorexia, 4 (4.6%) had jaundice, and 3 (3.5%) had vomiting. Clinical signs did not correlate with types of histological diagnoses.

Histopathologic distributions of canine hepatobiliary diseases from the present study are shown in Figure 1. Of the 87 canine biopsy cases, chronic hepatitis was the most common liver histopathologic finding (33; 37.9%). Other common findings included liver fibrosis (17; 19.5%), vacuolar hepatopathy (9; 10.3%), cholangiohepatitis (8; 9.2%), hepatocellular carcinoma (4; 4.6%), cholangitis (3; 3.4%), cholangiocarcinoma (2; 2.3%), and congestive hepatopathy (2; 2.3%). In addition, bacterial hepatitis (1; 1.2%), purulent necrotizing hepatitis (1; 1.2%), hepatic necrosis (1; 1.2%), hemangiosarcoma (1; 1.2%), hepatic carcinoid (1; 1.2%), bile duct

Table II. Dog characteristics according to the histological groups.

Histological groups	Number (%)	Median body weight (kg) (minimum, maximum)	Median age (y) (min, max)	Male:Female
Inflammatory response	63 (72.4%)	8.6 (1.9, 43.0)	10.5 (1.8, 16.5)	27:36
Regressive change	10 (11.5%)	6.4 (4.4, 24.6)	10.8 (4.2, 15.0)	6:4
Circulatory disturbance	3 (3.5%)	10.0 (3.0, 27.2)	10.3 (8.8, 15.0)	2:1
Tumor	11 (12.6%)	16.6 (4.5, 31.0)	11.3 (1.7, 14.1)	4:7
Total	87 (100%)	9.7 (1.9, 43.0)	10.6 (1.7, 16.5)	39:48

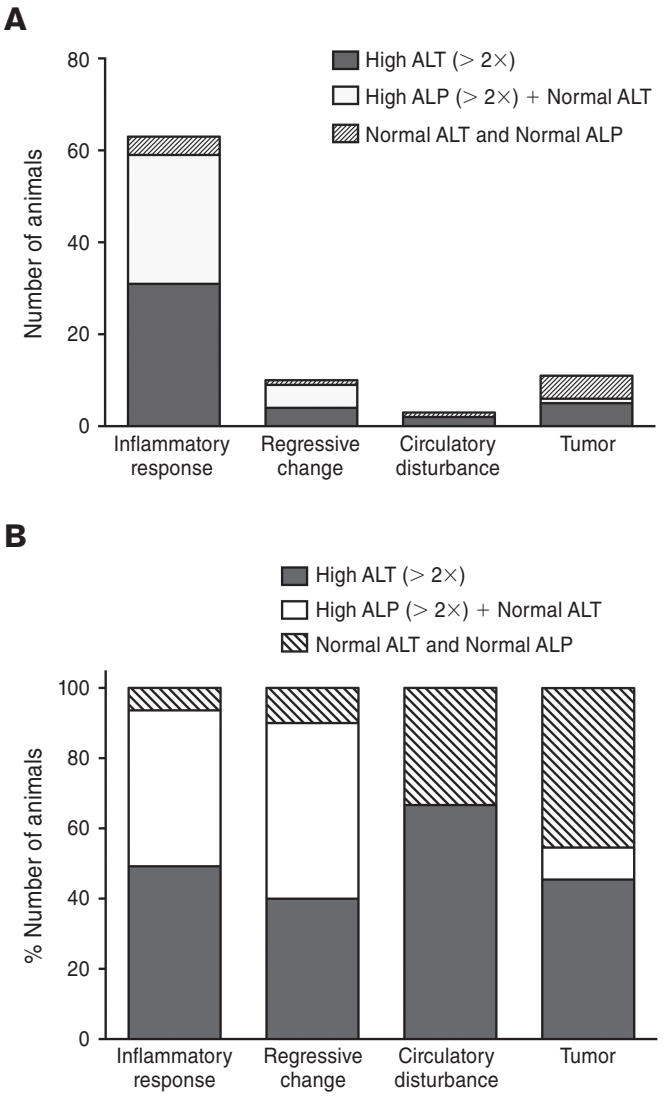

Figure 2. Percentage of animals categorized according to biochemical value of alanine aminotransferase (ALT) and alkaline phosphatase (ALP). A — Number of animals identified in each disease group. B — Percentage of animals diagnosed by biochemical markers in each disease group.

cystadenoma (1; 1.2%), hepatoblastoma (1; 1.2%), hepatoma (1; 1.2%), and sinusoidal ectasia (1; 1.2%) were identified.

The 3 most sensitive serum biochemical abnormalities in dogs with liver pathologies in the present study were elevated ALP (67; 77.0%), elevated GGT (47; 55.3%), and elevated ALT (42; 48.3%) (Table I). Interestingly, the number of patients with abnormal ALP was significantly higher than the number of those with abnormal ALT ($P < 0.001$), whereas the number of dogs with abnormal GGT compared with the number of those with abnormal ALT was not significantly different ($P = 0.365$). Other serum biochemical abnormalities included elevated preprandial bile acid (28; 33.7%) and hyperglobulinemia (26; 30.0%). Thrombocytopenia and anemia were identified in 16 (18.4%) and 14 (16.1%) dogs, respectively (Table I).

To evaluate the potential of ALT and ALP as markers for canine liver diseases, we categorized histological diagnoses into 4 groups (19): inflammatory diseases (63; 72.4%), neoplasia (11; 12.6%), regressive change (10; 11.5%), and circulatory disturbance (3; 3.5%) (Table II, Figure 2A). The results revealed that only 48.3% of dogs in all 4 disease categories had greater than 2-fold elevation of serum ALT (Figure 2B). In addition, elevation of ALP alone was enough to detect 40% to 50% of canine liver diseases with inflammatory and regressive change. Of the 34 patients with significantly increased ALP without elevated ALT, 28 (80%) and 5 (15%) had inflammatory liver diseases and regressive change of the liver, respectively (Figure 2A). In contrast, 35% to 45% of patients with circulatory disturbance disorders and tumors did not show significantly abnormal liver enzymes (Figure 2B). Elevation of ALT and/or ALP had a 90% and 93.7% sensitivity for diagnosis of canine inflammatory and regressive liver diseases, respectively, but only a 54.5% and 66.7% sensitivity for diagnosis of tumors and circulatory disturbance disorders, respectively (Table III).

ALT had a high degree of correlation with AST ($r = 0.938$, $P < 0.01$) and bilirubin concentration ($r = 0.714$, $P < 0.01$). ALT had a moderate degree of correlation with ALP ($r = 0.699$, $P < 0.01$) (Table IV). ALP had a high degree of correlation with AST ($r = 0.723$, $P < 0.01$) and bilirubin concentration ($r = 0.837$, $P < 0.01$). The cholestasis marker GGT had a moderate degree of correlation with ALP ($r = 0.670$,

Table III. Comparisons of the sensitivity of abnormal hepatocellular and cholestasis markers of liver diseases for detecting histopathologic changes. *P*-value compared to inflammatory response.

Histological groups	Number of dogs with high ALT and/or ALP > 2×	Number of dogs with normal ALT and ALP	Sensitivity (%)	*P*-value
Inflammatory response	59	4	93.7	—
Regressive change	9	1	90.0	0.532
Circulatory disturbance	2	1	66.7	0.214
Tumor	6	5	54.5	0.003
Total	76	11	87.4	0.161

ALT — alanine aminotransferase; ALP — alkaline phosphatase.

$P < 0.01$); however, GGT had a low degree of correlation with ALT ($r = 0.327$, $P < 0.01$) (Table IV).

Discussion

Our study was the first survey of canine liver diseases in Thailand. We demonstrated that the most common canine liver disease was chronic hepatitis (37.9%) (Figure 1). Warren-Smith et al (14) reported similar results, finding that 21% of dogs surveyed in their study had chronic hepatitis. However, microvascular dysplasia (29.4%) was the most common canine liver disease found in a prevalence study in Japan, with only 5.5% of dogs having chronic hepatitis (20). The difference could be due to breed and environmental factors.

Cholangitis is the most common hepatobiliary disease in cats (22). While the term cholangiohepatitis has been used in place of cholangitis, The term cholangitis is preferred because parenchyma involvement is often an extension of primary cholangitis (23). Recently, cholangitis/cholangiohepatitis in dogs appeared to be more common than previously thought (23). The prevalence of cholangitis/cholangiohepatitis was 12.6% in the present study (Figure 1) and 14.8% in Hirose et al (20). To the authors' knowledge, no other studies have reported the prevalence of canine cholangitis.

Although patients with increased liver enzymes due to extrahepatic causes were excluded in the present study, vacuolar hepatopathy, which is likely to be a secondary reactive change of the liver to other diseases, was a histological diagnosis. Vacuolar hepatopathy or vacuolar degeneration presented in 10.3% of the dogs in this study (Figure 1) and has been found in 10% to 19% of samples from other reports (14,20,24). Vacuolar hepatopathy is known to be associated with exogenous or endogenous glucocorticoid excess. Nevertheless, Sepesy et al (24) reported that 45% of dogs with vacuolar hepatopathy had no evidence of overt glucocorticoid exposure. Stress-induced hypercortisolemia as a result of an underlying primary disease may contribute to the development of vacuolar hepatopathy (24). Although high liver enzyme activity may be indicative of primary liver diseases and the need for further investigation using histology, vacuolar hepatopathy may be found in individuals with nonprimary liver diseases. In addition, vacuolar hepatopathy should not be ignored because fulminant hepatic dysfunction can be developed secondary to severe vacuolar hepatopathy (24).

Dogs with bacterial and purulent necrotizing hepatitis that are suspected of having a systemic infection were excluded from this study. However, we did not find clear evidence of systemic infection in these cases. It could be that the infection was localized only in the liver; this would explain how the dog with purulent necrotizing hepatitis fully recovered after its liver lobe with a necrotic lesion was removed. The dog with bacterial hepatitis had no clinical signs and presented only with chronic elevated liver enzymes. Bacterial hepatitis in this case may have been associated with cholangitis/cholangiohepatitis.

The prevalence of hepatocellular carcinoma (HCC) was previously reported as 35% and 52% of total primary hepatic neoplasms without breed predisposition (20,25). In the present study, the prevalence of HCC was 36.4% of total primary hepatic neoplasms (Figure 1). We found that HCC was more common than hepatoma, which is consistent with some previous studies (26–28). This result was inconsistent with the study in Japan, where there was no predominant prevalence of malignant and benign tumors (20).

Increased liver enzyme activity is often clinically associated with liver diseases when there is a 2-fold to 3-fold elevation of the upper limit of the reference range (8). Indications for liver biopsy in canines are often focused on chronic elevation of ALT (3). We compared types of abnormal liver enzymes, hepatocyte injuries, or cholestatic markers with histopathologic diagnoses. Our results showed that ALT was useful for diagnosing only 40% to 65% of all canine liver diseases. Similar results have been demonstrated in previous studies. The sensitivity of ALT is 45% for acute hepatitis in Labrador retrievers (29), 30% for passive congestion of the liver, 60% for cholestatic disorders, and 75% for chronic hepatitis (7). Interestingly, normal ALT activity can also be found in humans with hepatitis B and C viral infections (30–33). Patients with liver diseases such as advanced cirrhosis and those in the very early stages of a disease may not have significantly elevated ALT activity because they have fewer hepatocytes (34). Therefore, normal ALT alone should not be considered as an exclusion criterion for liver biopsy. Medical history, clinical signs, other liver enzyme activity, and ultrasonographic changes should also be taken into account. A marker of hepatocyte injury that is more sensitive than ALT is also needed to detect liver diseases early. One study revealed that microRNA-122 was more sensitive than ALT in identifying copper-induced hepatocellular injury (35); however, its use in clinical settings needs further study.

ALP and GGT are markers of cholestasis. In previous research, the highest levels of ALP developed in dogs with cholestasis, steroid

Table IV. Pearson's correlation coefficient *(r)* of different liver biochemistry measures.

Parameters	AST	ALP	GGT	Bile acid	Albumin	Bilirubin	PT	aPTT
ALT	0.938**	0.699**	0.327**	0.308**	0.022	0.714**	0.292**	−0.041
	(0.907, 0.959)	(0.573, 0.793)	(0.123, 0.505)	(0.099, 0.491)	(−0.190, 0.231)	(0.591, 0.804)	(0.085, 0.474)	(−0.250, 0.173)
AST		0.723**	0.380**	0.306**	−0.079	0.796**	0.318**	0.047
		(0.604, 0.811)	(0.181, 0.548)	(0.096, 0.489)	(−0.286, 0.135)	(0.703, 0.862)	(0.112, 0.497)	(−0.168, 0.258)
ALP			0.670**	0.276*	0.070	0.837**	0.234*	−0.005
			(0.533, 0.773)	(0.064, 0.464)	(−0.142, 0.277)	(0.760, 0.891)	(0.023, 0.425)	(−0.217, 0.207)
GGT				0.283**	−0.208	0.782**	0.201	0.167
				(0.070, 0.471)	(−0.403, 0.006)	(0.683, 0.853)	(−0.013, 0.399)	(−0.049, 0.368)

ALT — alanine aminotransferase; AST — aspartate aminotransferase; ALP — alkaline phosphatase; GGT — gamma-glutamyl transferase; PT — prothrombin time; aPTT — activated partial thromboplastin time.

* $P < 0.05$.

** $P < 0.01$.

hepatopathy, chronic hepatitis, and hepatic necrosis (36). Animals in our study showed a high incidence of elevated ALP and GGT, although the number of animals with abnormal GGT was not significantly different from that of animals with abnormal ALT (Table I). We found that 80% of animals with a greater than 2-fold elevation of ALP without significant elevation of ALT had inflammatory liver diseases (Figure 2A). Only 15% of those cases were diagnosed as a regressive change, which is likely to be a secondary change in the liver (Figure 2A). Almost half of dogs with high serum ALP activity did not show any evidence of overt glucocorticoid exposure (24). Moreover, glucocorticoid-induced ALP can be increased in liver diseases (34). In humans, hepatitis B viral infections can present with normal serum ALT but high ALP (37). Therefore, we recommend that dogs with unexplained significant elevation of ALP alone also be considered for liver biopsy.

About 90% of the dogs with inflammatory liver diseases and regressive change had significant elevation of ALT and/or ALP (Table III, Figure 2B). However, about 35% to 45% of patients with circulatory disturbance or tumors did not have elevated ALT or ALP (Figure 2B). This finding indicates that ALT and/or ALP had a high sensitivity (93.7% and 90%, respectively) for detecting diseases involving inflammation and regression but a low sensitivity (66.7% and 54.5%, respectively) for detecting circulatory disturbance or tumors (Table III). It has been known that increases in a single liver enzyme, especially a mild elevation, may be nonspecific for liver diseases (8) and that caution should be used when interpreting the results for canine patients with less than a 2-fold elevation of liver enzymes. Therefore, liver enzyme activity should be interpreted together with clinical signs and liver ultrasonographic appearance, especially when diagnosing primary liver tumors.

A high degree of correlation was identified between hepatic injury markers ALT and AST (Table IV). The cholestasis markers, ALP and GGT, were also moderately associated with each other. Our correlation studies also revealed a relationship between hepatic injury markers (ALT and AST) and cholestasis markers (ALP and GGT) (Table IV). Among various hepatic function tests, bilirubin concentration was highly correlated with hepatic injury markers as well as cholestasis markers.

Our study had several limitations. Reliable histopathologic results depend on a liver sample of adequate size and quality (38,39). The use of the needle biopsy technique in this study may have resulted in the collection of inadequate specimens and may not have represented all lobes of the liver (4,6). Caution should be taken when interpreting histopathologic findings from needle biopsy specimens (6); therefore, we took a biopsy from 2 different liver lobes to increase the likelihood of obtaining a sample that reflected the predominant histologic abnormality, as recommended (4). Moreover, comparison of 8 mm punch, 5 mm laparoscopic cup, and 14-gauge needle samples gave a similar proportion of samples in agreement with standard wedge biopsy samples. Obtaining multiple samples from the liver might be more important than the method of biopsy (4). Another limitation is that the interpretation of biopsy specimens is subject to significant interobserver variation — ideally, more than one pathologist should evaluate each section (3). The present study relied on a single pathologist for histopathologic interpretation of all the liver samples, so the results may incorporate bias in the pathology report. On the

other hand, a single pathologist likely produced more consistent findings than would have multiple observers (40).

This study was the first to quantify the prevalence of histological diagnosis of canine liver diseases in Thailand and we revealed a pattern of liver enzyme abnormalities in each disease category. We demonstrated that there were limitations to the use of serum liver enzyme activity for the diagnosis of canine liver diseases. We suggest that patient history, clinical signs, both hepatic injury and cholestasis enzymes, ultrasonographic features, and the possibility for other nonprimary liver diseases be taken into consideration before performing a liver biopsy in individual patients.

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