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ORIGINAL ARTICLE

Quantitative ultrasound elastography and serum ferritin level in dogs with liver tumors

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ABSTRACT

Objective: The objective of this study was to assess the serum ferritin level and quantitate ultrasound elastography as a marker to distinguish dogs with benign and malignant liver tumors.
Materials and Methods: Twenty-eight dogs were determined the serum ferritin and ultrasound elastography by using fine-needle aspiration biopsy.
Results: Our results demonstrated that dogs with malignant liver tumors had significantly higher mean serum ferritin concentrations than those with benign liver tumors (p = 0.004). The mean intensity of blue and red colors from elastography was greater in the malignant than those in the benign group, especially for the blue color, meaning that lesions showed more hard tissue. Additionally, histograms of blue color in the malignant tended to be higher than the benign group.
Conclusion: We suggested that quantitative ultrasound elastography and serum ferritin concentration comprise an alternative and non-invasive diagnostic method that could be used to predict the type of liver tumors in dogs.

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Introduction

Liver tumors typically occur in older animals and may be classified as primary liver tumors or metastatic liver tumors. The incidence of the primary liver tumor is 0.6%–1.5% for all canine tumors, while metastatic are three times more frequent than primary liver tumors and possibly to develop the malignant [1]. The most primary liver tumor is hepatocellular carcinoma in dogs, whereas most metastatic liver tumors originate from multiple visceral organs, including the spleen, pancreas, and intestinal tract [2]. Dogs with liver tumors can be asymptomatic or reveal nausea or vomiting, weight loss, loss of appetite, diarrhea, or abdominal distention [3].

The diagnosis of liver tumors is usually performed by abdominal imaging (X-rays and ultrasound). However, it does not provide enough information. Blood tests are not specific for these tumors, but they can reveal signs of liver injury and bile duct obstruction. Liver biopsy and fine-needle aspiration biopsy (FNAB) remain the gold standard for the diagnosis of liver tumors [4,5]. The major complication for this procedure is bleeding, which occurs in 6% of sampling cases [4].

Ultrasonographic elastography is a method used for the visualization of tissue elasticity and stiffness [6]. It is a non-invasive method developed for being effective and safe to evaluate liver tumors [7]. Real-time tissue elastography has already been utilized for the diagnosis of breast cancer [8], thyroid cancer [9], lymph nodes [10], and prostate cancer [11]. Previous reports for ultrasound elastography suggested that patients with higher hepatic elasticity were much more likely to develop liver cancer, portal hypertension, gastric and esophageal varices or hemorrhage, hepatic decompensation, and mortality [12,13].

A tumor marker is an indicator for evaluating the incidence of a neoplastic process [14]. Hyperferritinemia can be found in dogs with immune-mediated hemolytic anemia,

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canine histiocytic sarcoma, liver disease, lymphoma, and inflammation [15]. It may reflect lasting necroinflammatory goings-on, and often complements with iron accumulation in hepatic mesenchyme cells [16]. A previous study showed that elevated ferritin levels were associated with hepatic injury [17]. The additional study indicated that the ultrasound elastography results in significant accuracy [18]. Screening with ultrasound elastography can identify the disease at the initial stage, and mediation could be started.

It remains unclear whether real-time tissue elastography affords for veterinary clinical on the diagnosis of tumors in liver dogs. Therefore, this study was to measure the serum ferritin level and quantitate ultrasonographic elastography to differentiate dogs with benign and malignant tumors. The results will advantageous for dogs of liver tumors in disease progression and help in medication preparation. Therefore, ultrasound elastography is helpful in the prediction of prognosis. Non-invasive ultrasound elastography will be much more widely used clinically in the future of veterinary diagnostics.

Methods

Animals

Thirty healthy and hepatic tumor dogs were incorporated in this study from Prasuarthorn Animal Hospital. The dogs consisted of males and females ranging in age more than 6-year old and weighing more than 1 kg. The protocol was approved by the Animal Ethics Committee at the Faculty of Veterinary Science, Mahidol University of Thailand (MUVS-2016-04-18).

Thirty healthy dogs had to be in good health, be less than 6-year old, not be limited to gender, and not have a history or signs of hepatic disease. Furthermore, the results of the physical (vital sign) system had to be normal.

Twenty dogs were both genders with hepatic tumors, more than 6-year old of age, weighing more than 1 kg, and having a history of hepatic disease. The results of physical examination and laboratory results will have the criteria for the selection of dogs with hepatic tumors as follows: inclusion criteria comprising (1) increased liver enzyme level (2) focal lesion in hepatic ultrasonography; exclusion criteria comprising (1) increased liver enzyme level but no focal lesion in hepatic ultrasonography identified.

When all data was collected, an appointment with the owner of the dog was made for blood collection to evaluate blood ferritin level and perform hepatic ultrasonographic elastography as well as fine needle biopsy at Prasuarthorn Animal Hospital.

Ultrasonographic elastography

Elastography ultrasound examinations were performed using a logiq P6 with an real-time elastography (RTE) module (GE Healthcare, Thailand). Dogs were given an anesthetic injection with diazepam 0.1–0.5 mg/kg ip. and tramadol 2–4 mg/kg, keep intravenous (IV) fluid (Acetar Ringer's solution) rate 10 ml/kg/h, give cefalexin antibiotic 15–30 mg/kg for subcutaneous prophylaxis route and then inject propofol 5 mg/kg IV, monitoring and maintaining the depth of the anesthesia of dogs. The dogs were placed in a ventrodorsal position.

Conventional ultrasound examination in regular anatomical B-mode imaging and ultrasound guide FNAB, strain elastography ultrasound were performed in the dogs for the collection of data from all dogs. The results from ultrasound-fine needle aspiration (US-FNA) cytology were provided by a pathologist.

Conventional ultrasound, ultrasound-guide FNAB, and ultrasound elastography were achieved with a GE Healthcare logiq P6. The depth of the located target mass in the liver was less than 5 cm by using conventional U/S and US elastography examination. All characteristics of the liver mass images were described for size, echogenicity, structure, and shape. All ultrasound-guide FNAB procedures were carried out by using 22G, single-use biopsy needles with at least one puncture, and continuous push and pull fashion without suction. Ultrasound elastography was performed during an ultrasound examination with a movie of 10–20 sec recorded. The frequency of ultrasound elastography was set at 10–12 MHz. A veterinarian monitored all the procedures.

Serum samples for measurement of ferritin were collected concurrently with complete blood count (CBC) and serum biochemistry by using a 22G needle. Serum ferritin samples were stored at −70°C until they were sent in batch to the Central Laboratory for analysis. Canine ferritin level was measured by the enzyme-linked immunosorbent assays technique.

Quantitative elastosonography image analysis

The visualized tissue elasticity patterns and different elasticity values are marked with different colors. US elastography is presented in red-green-blue (RGB), whereas hard tissue was marked with blue. Intermediate tissue hardness was marked with green and soft tissue was marked with red. Elastosonography images in digital imaging were exported into Image J software to achieve this study. The process of image analysis was following in Landoni et al. [8].

The histogram was analyzed for the region of the liver's mass in five pictures. The region of interest (ROI) was determined by the entire area of mass, which was located in the region on a grey scale from regular anatomical B-mode imaging. The same ROI was copied onto an observed area in the US elastography image. The histogram analysis was applied to the color mode. The total mean histogram of the region was calculated from each picture for all dogs.

Obtained elasticity images were motion images. For each elastography film, 15-sec long (about 120 frames) frames were selected by a green bar of the pressure gauge. The evaluation of quality for the elasticity images and diagnostic score of elasticity was performed by logic P6 with an RTE module (GE Healthcare, Thailand). Each elastosonographic image was analyzed by using Image J, developed at the National Institute of Health, Maryland, the USA for which a special dynamic analysis plug-in was created. Elastography film was evaluated the color information concerning the region to differentiate between benign and malignant liver lesions (Fig. 1B and C).

Statistical analysis

It was performed with IBM Statistical Package for the Social Sciences software version 19.0 (Armonk, NY). For the hepatic benign and malignant tumor dog, the mean and standard deviation were calculated and histogram analyses of ROI. The mean serum ferritin level and histogram analysis were compared between the benign and malignant tumor dog using the Mann–Whitney U-test. Statistical significance was set at a $p < 0.05$.

Results

Clinical characteristics of healthy, benign and malignant dogs

Twenty-eight dogs were accepted for participation in this study, comprising seven healthy dogs, 10 with benign tumors, and 11 with malignant tumors. The eight dogs with benign tumors had liver haemangioma consisting of a group of six females and two males. Ten malignant dogs group included seven females and three males. The following breeds were represented: Golden Retriever (1), Mixed-breed (8), Shih Tzu (1), Poodle (3), Beagle (1), Siberian Husky (1), Pommerinian (1) Cocker (1), and Miniature Schnauzer (2). The summary of characteristics data and blood profiles for the dogs is presented in Table 1.

The malignant lesions included metastasis carcinoma hepatitis, hepatitis and carcinoma, malignant epithelial tumor, hepatocellular carcinoma, bile duct neoplasia, and hepatic carcinoma. The benign lesions included hepatitis, inflammation, hepatic nodular hyperplasia, and hepatic cirrhosis. General data cytology and liver ultrasound findings of the benign and malignant dogs are presented in Table 2. There were no significant differences in age or blood profiles between the benign and malignant dogs.

Serum ferritin levels

The dogs with malignant tumors had a mean serum ferritin levels of 21.51 ± 8.28 ng/ml, whereas the dogs with benign were 6.66 ± 7.23 ng/ml. The mean serum ferritin levels were significantly different between benign and malignant dogs ($p = 0.004$). Serum ferritin values in dogs with benign and malignant are shown in Table 3.

Quantitative elastography images

The diagnosis of all malignant lesions was confirmed based on the pathologic findings. Ultrasound-guided aspiration of the liver was performed in 18 dogs. None of the dogs experienced complications secondary to ultrasound-guided aspiration (Figs. 1A, 3A and B).

Regions of softness, with a lower number of blue counts, relate to higher values of stiffer tissue, whereas regions that are fewer resistant to pressure with a higher number of red counts relate to higher values of softness. Our results showed that tumor lesions were blue, meaning hard tissue, while the surrounding fatty tissue was red, meaning soft tissue.

The average histogram obtained by analyzing the elastosonographic image of blue, green, and red colors from the US images of the nodules was 8,582, 7,727, 7,808 in the malignant group, and 7,020, 9,289, 5,691 in the benign group, respectively. There were no significant differences between the presence of malignant and benign groups from the Mann–Whitney test with $p = 0.518$, $p = 0.247$, and $p = 0.518$ for blue, green, and red, respectively.

Blue colors had histogram values in the malignant group more than the benign group, however, meaning that lesions in the malignant group showed hard tissue more often than the benign group. According to the green color, histogram values in the benign group were more than the malignant group, meaning that lesions in the benign group showed more soft tissue than the malignant group. The boxplot color histograms of red, green, and blue counts inside the ROI for the lesions are shown in Figure 2.

Color histograms of red, green, and blue counts inside the ROI and ratio blue/green were calculated in percentage in total (%) for all the benign and malignant lesions, as shown in Table 4. The mean ratio of blue/green between the benign and malignant groups showed a significant difference ($p = 0.004$).

Discussion

Our results showed that hyperferritinemia was found in dogs with malignant liver tumors. We found the values of serum ferritin in benign and malignant tumors were in the upper reference limit. The mean values of the intensity of red, green, and blue colors were higher in the malignant group more than the benign group. We found a positive correlation between serum ferritin and elastography images in dogs with liver tumors.

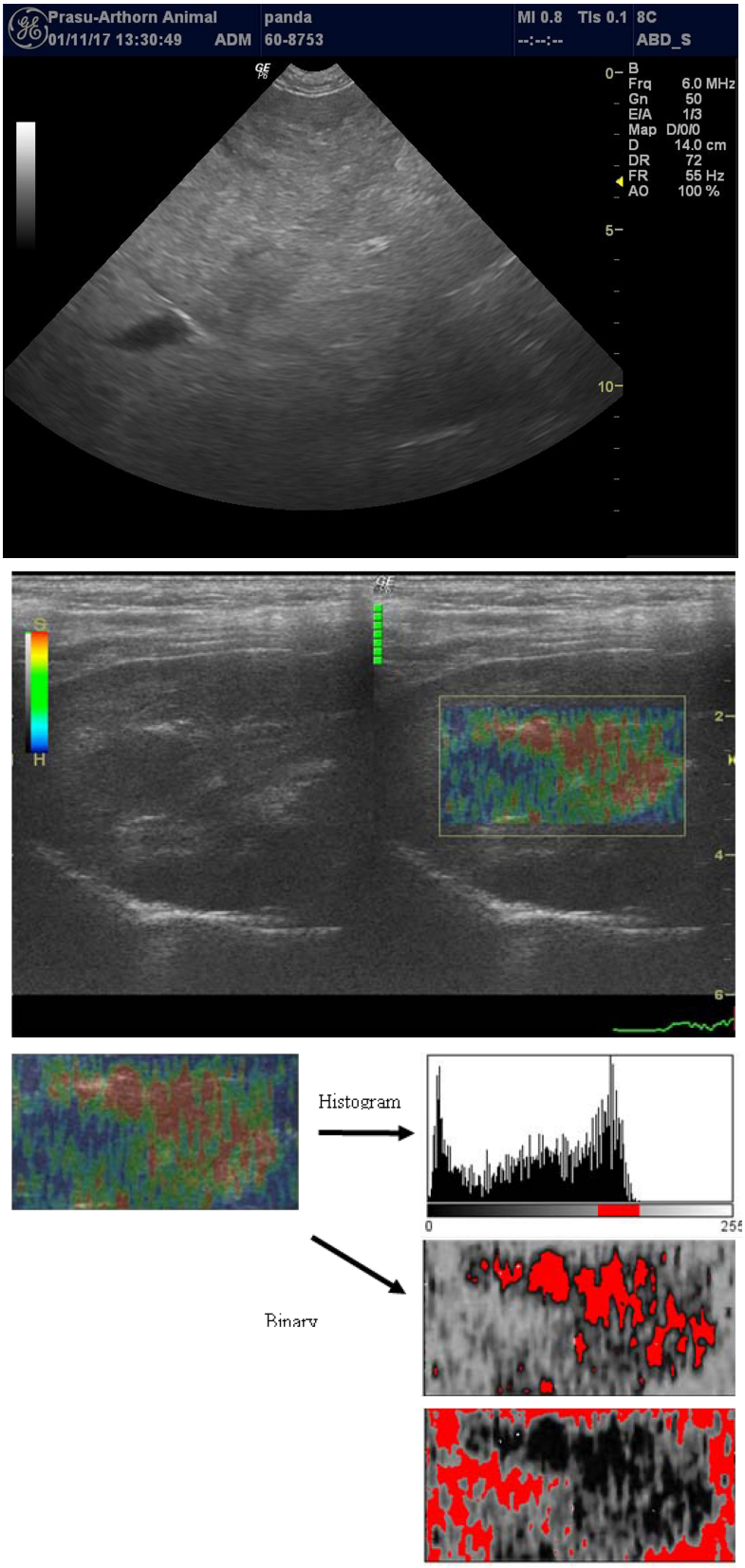

Figure 1. (A) Normal liver parenchyma in conventional ultrasound B-mode. (B) The strain elastography of the liver was displaced in the B-mode image as a dual-screen mode. (C) Image J software with the image and the analysis color histogram of red, green, and blue.

Table 1. Characteristics, blood, liver enzyme profiles of benign and malignant liver tumor dogs.

Variables		All type	Benign	Malignant
		Mean ± SD		
Age	Year	7.53 ± 3.69	7.67 ± 3.06	7.35 ± 2.79
Gender	Male	13	6	7
	Female	5	2	3
Breed	Domestic	8	3	5
	Other	10	7	4
ALP (U/L)	Ref. ranges 23–212	1,858.78 ± 2,571.34	2,463.11 ± 3,489.80	1,254.44 ± 1,024.50
ALT (U/L)	10–100	247.00 ± 234.71	214.33 ± 180.26	279.67 ± 286.65
Plasma Protein	6.0–7.5	9.03 ± 1.07	9.50 ± 0.82	8.55 ± 1.12
Albumin (gm/dl)	2.7–3.8	2.64 ± 0.77	3.2 ± 0.99	2.27 ± 0.40

ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; No. = Number.

Table 2. Data cytology and liver ultrasound finding of benign and malignant liver tumor dogs.

No.	Group	Sex	Age (month)	Cytology	Liver ultrasound finding
1	Benign group	M	144	Suppurative hepatitis	Hepatic mass , Heterogenous parenchyma with mixed echogenic mass in all liver lobes
2		F	163	Inflammation	6 cm diameter, focal, mixed echogenicity mass at caudate lobe of liver, hepatic lymph node enlargement
3		M	145	Hepatic adenoma	Hepatogenous with mixed echogenic parenchyma in all liver lobes
4		M	144	Chronic liver disease	2 cm diameter, diffuse, hyperechoic nodule along with heterogenous liver parenchyma in all liver lobes and cholangitis
5		M	121	Hepatic nodular hyperplasia, hepatic cirrhosis	Hepatic nodular hyperplasia, vacuolar hepatopathy
6		M	132	Suppurative hepatitis	Hepatic mass, Heterogenous parenchyma with mixed echogenic mass
7		M	156	Suppurative hepatitis	Homogenous diffuse hypoechoic with rounding at caudal tip (Hepatitis)
8		F	132	Hepatic adenoma	Hypoechoic of liver parenchyma with prominent hepatic vein, cholangiohepatitis or amyloidosis
9	Malignant group	M	154	Hepatic carcinoma	12 cm diameter, focal, mixed echogenicity mass at right lobe. Hepatic lymph node enlarge with the same echogenicity
10		M	144	Metastasis carcinoma hepatitis	Heterogeneous parenchyma with mixed echogenic mass at right liver lobes with irregular of liver contour (Hepatic mass carcinoma/nodular hyperplasia/ lymphoma)
11		M	105	Cholangiocellular carcinoma or hepatocellular carcinoma	Hepatic mass in all lobes (carcinoma, nodular hyperplasia, infiltrative lymphoma)
12		F	156	Hepatic nodular hyperplasia, hepatitis and carcinoma	5 cm diameter, hyperplasia, calcified, granuloma, mixed echogenicity mass at right lobe
13		F	77	Malignant epithelial tumor, hepatocellular carcinoma, bile dict neoplasia	Hepatic mass, carcinoma, metastasis
14		M	127	Hepatic carcinoma	Chronic hepatitis (between cirrhosis), cholecystitis/hypoalbuminemia
15		F	180	Hepatic carcinoma	Primary carcinoma, hyperplasia (non-neoplastic), metastatic.
16		M	108	Hepatic carcinoma	Hepatic mass, carcinoma, metastasis
17		M	96	Hepatic carcinoma	Lymphoma infiltrative, carcinoma, nodular degenerative
18		M	144	Hepatic carcinoma	Heterogenous of liver parenchyma with multiple hypoechoic and mixed echogenic nodular lesions in all liver lobes (Lymphoma infiltrative, metastasis, carcinoma, nodular hyperplasia)

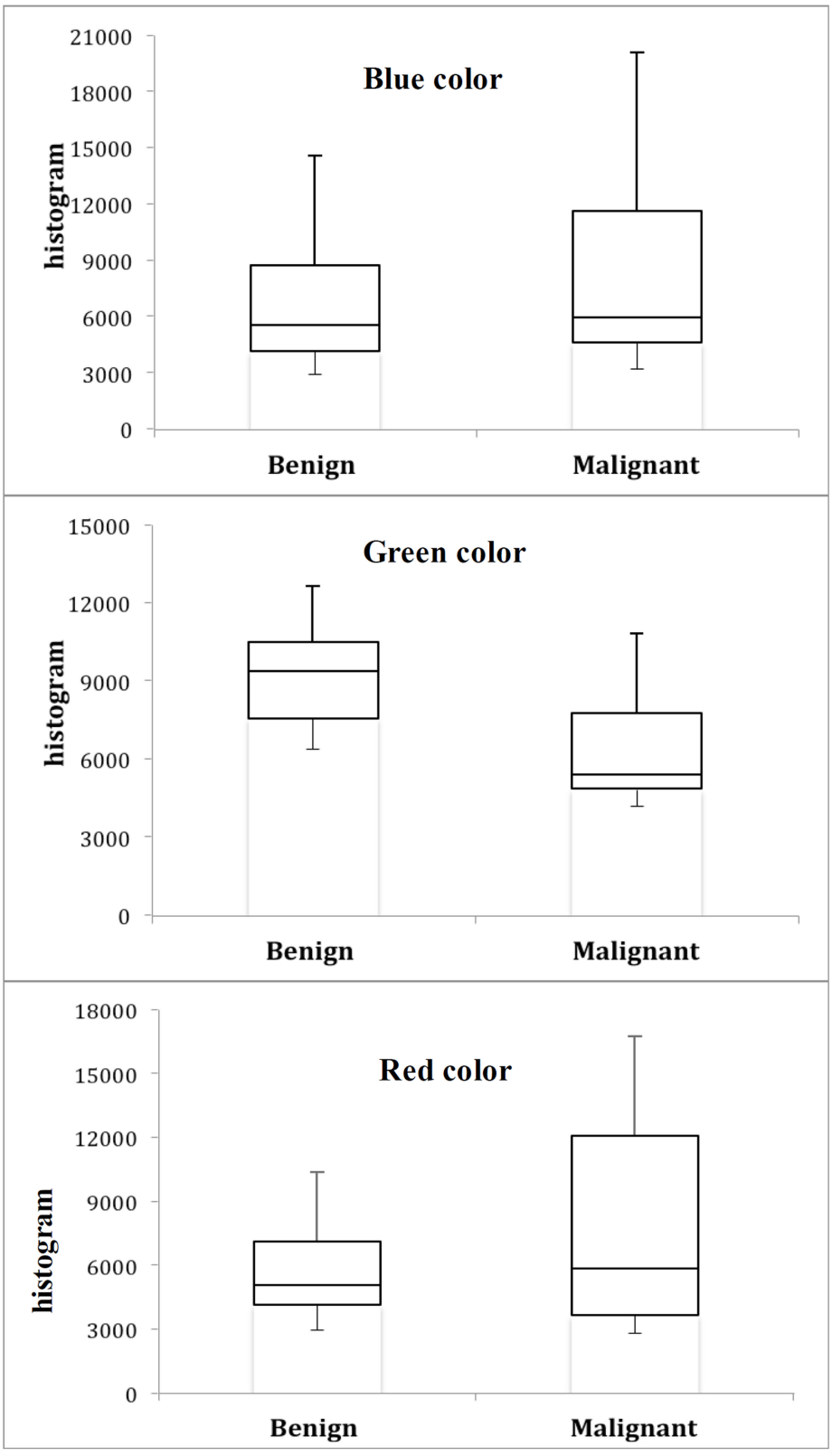

Figure 2. Box plots of quantitative histogram analysis of elastography results for blue, green, and red intensity colors for the benign and malignant liver dogs.

The dogs with liver neoplasia present with non-specific signs. They have clinical signs that could be attributed to their liver tumor mass [19]. Historical abnormalities include lethargy, inappetence, weight loss, anorexia, diarrhea, polyuria polydipsia, seizure, melena, and ataxia [3]. The clinical approach and method for diagnosis of an

Table 3. Serum ferritin values in dogs with benign and malignant liver tumor.

Group	No.	Serum ferritinmean ± SD (ng/ml)	Range	*p*-value
Normal	7	0.05 ± 0.14	0.00–0.38	
Benign	8	6.66 ± 7.23	0.00–19.23	
Malignant	9	21.51 ± 8.28*	8.26–37.72	0.004*

Table 4. Color histogram of red, green and blue counts inside the ROI and ratio blue/green (%) for all the benign and malignant lesions.

Color	Benign	Malignant	*p*-value
	mean ± SD		
Blue	71.73 ± 8.6	77.00 ± 15.27	0.324
Green	88.77 ± 16.5	83.63 ± 12.46	0.594
Red	67.97 ± 7.87	75.35 ± 10.96	0.121
Ratio blue/green	0.80	0.92	0.044*

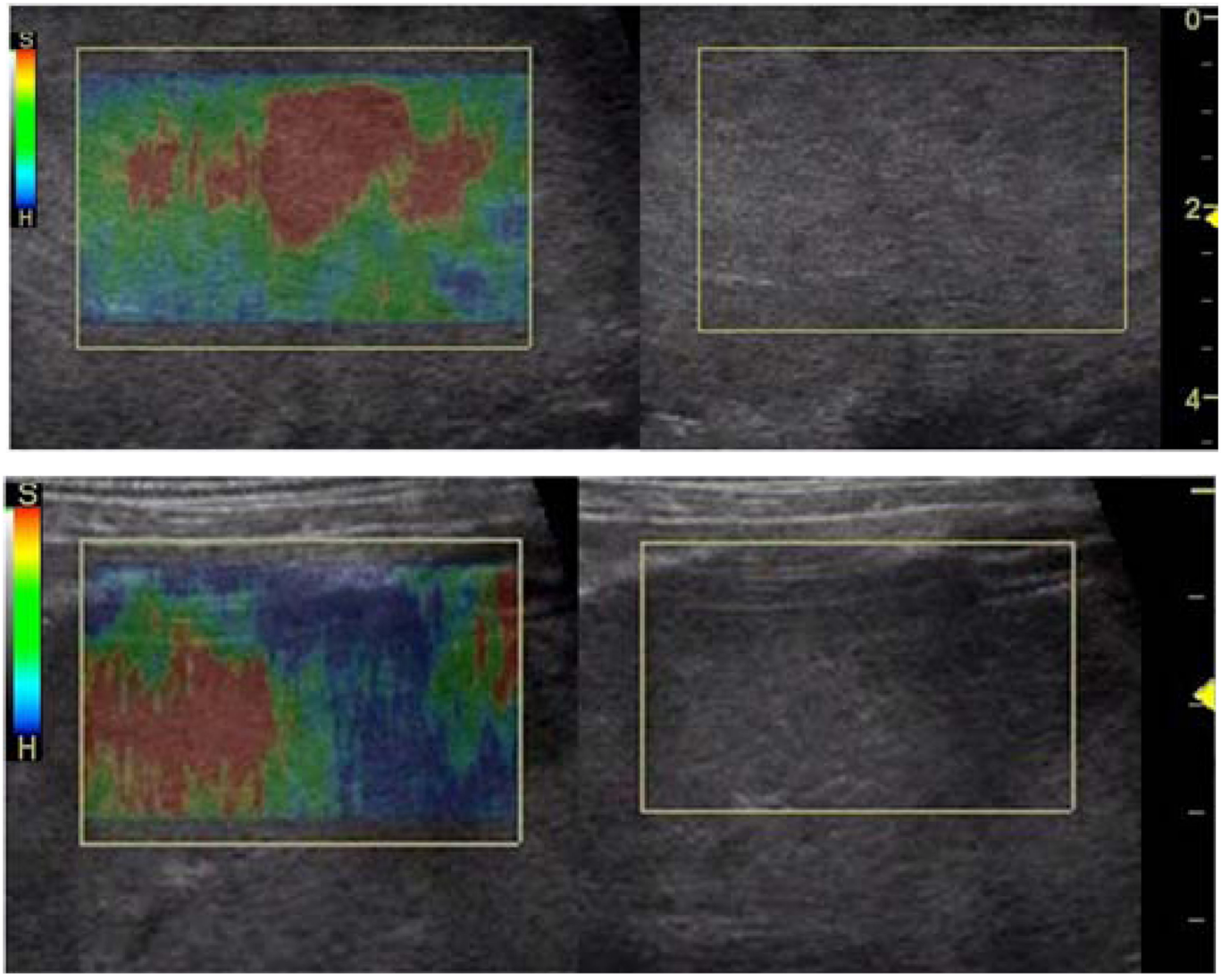

Figure 3. (A) Elastographic image of the benign liver tumor, the liver parenchyma exhibit a higher ratio of red and green color than blue color (left). The liver parenchyma is softer than the abdominal wall and shows a homogenous pattern that the echogenicity is similar to the normal liver parenchyma (right). (B) The elastographic image of the malignant liver tumor, the liver parenchyma, exhibits a higher ratio of blue color (left). The liver parenchyma is showing hyperechoic and exhibits a stiff homogenous pattern (right).

animal with suspected liver tumors include complete blood count, blood chemistry, coagulation test, urinary analysis, thoracic and abdominal radiographs, abdominal ultrasonography [19].

Liver biopsy is the gold standard for diagnosis of the staging of liver disease and is routinely achieved for the diagnosis of the lesions in the liver [20]. Ultrasound-guided biopsy has very high sensitivity and specificity [5]. The risk of malignant seeding during a biopsy is considered rare. Mortality and serious problems include bleeding episodes, hematomas, sepsis, pancreatitis, focal infection, pneumothorax, and hypotension [21] can be found and have to be considered when a biopsy is performed.

Abdominal ultrasound has an important role in the diagnosis of canine liver disease [22]. An elastography ultrasound is a non-invasive method that is used to access tissue elasticity and is easily applied to the canine liver [23]. Elastography ultrasound has demonstrated a tool to distinguish between benign and malignant lesions of the breast, prostate, thyroid gland, lymph nodes, and many other sites [24–26]. Therefore, this technique can detect small nodule lesions [8].

Histogram was used to evaluate the color intensity in the ROI for distinguishing between benign and malignant liver lesions [8]. In our study, there were no significant differences between the presence of malignant and benign groups. However, the histogram showed that malignant liver tumors had a higher blue color ratio in the image, meaning tissue stiffness is increased. It might be a possibility to predict malignant lesions. On the other hand, a higher red color ratio can conduct to a non-malignant or inflammatory lesion in dogs.

Ferritin is a globular protein containing iron, which is produced in the liver, spleen, and other organs. It mainly stores in the liver, spleen, bone marrow, and intestinal mucosa. Ferritin was first discovered in the 1930s, with many studies published several years thereafter [27].

In human medicine, studies have revealed that the elevation of serum ferritin level is associated with acute or chronic liver disease and in many malignancies. One report mentioned that primary hepatocellular carcinoma is significantly associated with elevated serum ferritin levels. In men, serum ferritin levels higher than 300 ng/ml, are caused by chronic liver disease and the development of primary hepatocellular carcinoma [28]. Another study suggested that renal cell carcinoma could cause the elevation of serum ferritin levels (552 ng/ml). It is not correlated with age or gender [29].

Mechanisms of increased serum ferritin include injuries to liver tissue, resulting in the release of ferritin into the bloodstream, which can be detected by increasing serum ferritin level, destruction of red blood cells, and malignancies [15]. Besides that, some studies suggest the possibility of ferritin, which gives to the etiology of cancer. The mechanisms are unclear, but there is a hypothesis explaining that ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}), which can catalyze superoxide and hydrogen peroxide into hydroxyl radicals. Hydroxy radical is an oxidizing agent that can inhibit the tumor suppressor gene and activate oncogene [27].

In veterinary medicine, serum ferritin can be used as a tumor marker for canine histiocytic sarcoma. It can differentiate between dogs with histiocytic sarcoma from dogs with inflammatory disease, liver disease, and lymphoma [15]. Our study shows that the elevation of serum ferritin is common in dogs with malignant liver tumors [89% of dogs with malignant liver tumors have high serum ferritin, while they are less common in dogs with benign liver tumors and control group (60% and 17%, respectively)]. The benefits of relating RTE for the diagnosis of liver tumors [30,31].

The main limitation of this study is that present RTE has limits in acquiring elasticity images of liver tumors extracorporeal [32]. Because it uses a high-frequency US probe, deep areas of the liver cannot be visualized. Furthermore, some parts of the liver cannot be compressed enough to induce the strain to obtain elasticity images through the thoracic-abdominal wall. Another drawback is the absence of a standardized quantitative measurement to correctly characterize the fusion of tissue hardness illustrated by elastography images. The quantitative histogram of the original colors RGB in images recorded during real-time transabdominal or endoscopic ultrasound (EUS) Elastography (Adobe PhotoShop 7.0; ImageJ) may be used instead of the qualitative, highly subjective, observatory dependent assessment of the tumoral pattern.

Future studies should seek to confirm these findings in randomized and more extensive studies to test the selected cut-offs for the mean intensity of green and blue colors for the diagnosis of hepatocellular carcinoma. Additionally, studies are needed to evaluate whether serum ferritin can help as a serum tumor marker, such as representing tumor prognosis, response to medication, and reappearance.

Conclusions

Our results demonstrate the RTE in the investigative of liver tumor suggest that US elastography is a method for the distinction of benign and malignant liver lesions. The findings show that it is valuable for the identification of characteristic benign and in the differentiation of malignancies. Ultrasonographic ultrasonography and serum ferritin level have great potential as a useful modality for the diagnosis of liver tumor dogs. However, the gathering and integration of historical and laboratory data are also needed to diagnose liver tumors. Nevertheless, liver

biopsy remains the gold standard for the diagnosis of liver tumors.

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Conflict of interest

The authors declare that no conflicts of interest in this paper.

Authors' contribution

This study was accomplished in participation between all authors. SH designed the study, selected a case study, conducted the experiment. PY analyzed serum ferritin, designed the analysis results, did statistical analysis, and prepared the manuscript. PP, MW, and AP, did data collection, quantitative elastography images, analyzed the hematological and biochemical parameters. PJ and KC scan ultrasound elastography. The manuscript was approved by the authors.

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