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Clinical studies on shear wave elastography (SWE) of liver in healthy dogs

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Shear wave elastography (SWE) has been documented as a useful tool for evaluating liver parenchyma in human medicine for diagnosis of chronic liver disorders including neoplasia. The aim of this study was to evaluate SWE of liver in healthy dogs. A total of 40 healthy dogs were included in this study. The most suitable acoustic windows for recording shear wave ultrasonography in dogs were established and documented on the basis of consistency of results, ease of recording SWE and animal compliance. The mean value of the SWE measurements of healthy liver in 40 dogs was 2.09±0.73 kPa for left liver lobes and 2.23±0.84 kPa for right liver lobes. The liver stiffness values obtained by SWE were not influenced by the gender and size of the animal. The mean value liver stiffness in dogs below 1 yr age group was significantly (Pd"0.01) lower than the dogs above 1 yr of age. It was concluded that left paracostal for left liver lobe in right lateral recumbency and right 11th-12th intercostal space for right liver lobe in left lateral recumbency were ideal windows for performing liver shear wave elastography in healthy dogs. The stiffness values of liver in healthy dogs range from 1.86-2.50 kPa.

***Key words**: Dog, Liver, Shear wave elastography (SWE)*

Elasticity elucidates the ability of a material or tissue to regain its original shape and size following application of stress. Elastography is a technique to evaluate the elasticity of a tissue and provides information about stiffness of tissues (Wells and Liang, 2011). Elastography is based on the principle that neoplastic tissues have increased cell density, resulting in reduced elasticity and therefore increased tissue stiffness. There are different methods for performing elastography which vary in the way that the force is applied to deform the tissues. These fall into two major categories called 'strain elastography' and 'shear wave elastography' (Ophir *et al.*, 2002). Shear wave elastography (SWE) relies on the generation of shear waves determined by the displacement of tissues induced by the force of a focused ultrasound beam (Goddi *et al.*, 2012). The propagation velocity of the shear waves correlates with the elasticity of tissue; i.e., it increases with increasing stiffness of the parenchyma (Sarvazyan *et al.*, 2011).

Ultrasonography has been accepted as one of the most popular non invasive diagnostic imaging technique for the detection of abnormalities in liver parenchyma. The diseases of liver are common in dogs. These diseases are generally diagnosed on the basis of history, physical examination, laboratory tests, and different diagnostic imaging tests such as radiography, ultrasonography, CT scan and MRI. However, biopsy of the lesion in the hepatic parenchyma is considered as gold standard for a conclusive diagnosis. In medical literature elastographic US-based technique has proven to be a promising and accurate tool for differentiating benign from neoplastic lesions in patients without liver cirrhosis (Conti *et al.*, 2016). The SWE also has an added advantage that it can facilitate quantitative assessment to the hepatic parenchyma. In humans the mean shear wave velocities have been reported for hepatocellular carcinomas (2.48 m/s), cholangiocellular carcinoma (1.65 m/s), metastases (2.35 m/s), hemangiomas (1.83 m/s), and focal nodular hyperplasia (0.97 m/s) (Park *et al.*, 2013). Another study in humans' liver elastography revealed the shear wave velocity (SWV) of malignant and benign masses were 2.95±1.00 m/s and 1.69±0.89 m/s, respectively which were significantly ($P \leq 0.001$) varied (Guo *et al.*, 2015). Many studies in human medicine graded diagnostic accuracy of elastography as fair (Guo *et al.*, 2015) to good (Zhang *et al.*, 2013; Sandulescu *et al.*, 2012), in confirming the liver malignancy.

Some experimental work is available in canines where shear wave elastography was used to evaluate liver stiffness of 15 dogs of different breed. The median liver shear wave velocity value observed in those dogs was 1.04-1.38 m/s (depending on depth) (Holdsworth *et al.*, 2014). To the authors knowledge no reports are available on reference data of stiffness values of liver by SWE in healthy dogs. The present study describes the technique, ideal windows and ideal recumbency for recording normal stiffness values of liver in healthy dogs by SWE.

Materials and Methods

The study was conducted on 40 apparently healthy dogs presented for routine vaccinations,

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deworming, elective castration/ovariohysterectomy. Necessary permission for conducting the trial was obtained from Institutional Animal Ethics Committee (IAEC) and due consent was taken from the animal owners prior to ultrasonography and elastography in dogs. All the dogs were subjected to detailed clinical and physical examination. The age, breed, gender and body weight of the dogs was recorded. Complete blood count and estimation of liver enzyme were carried out to rule out any liver involvement. Based on the above observation the animals were declared healthy. Along with these parameters, B-mode ultrasonography of liver was done to subjectively evaluate the liver parenchyma.

Four different anatomical windows each were examined for evaluating liver in all 40 dogs in order to find the ideal windows for carrying out SWE of liver. The animals were scanned in real time B-mode using 6-12 MHz linear array transducer for qualitative evaluation followed by shear wave elastography using 1-5 MHz convex array transducer of Philips Affinity 70 Ultrasound Machine.

To standardize the anatomical windows for liver, examination was done from the left 11^{th}-12^{th} intercostal space (LICS) and left paracostal (LP) for left liver lobes. For evaluation of the right liver lobes the right 11^{th}-12^{th} intercostal space (RICS) and right paracostal (RP) area was evaluated. Region of interest (ROI) box was placed within 6-7 cm distance from Glisson's capsule in the liver lobe devoid of large blood vessels. Ten repeated SWE measurements were acquired at each anatomical window. For obtaining consistent and accurate measurements of the stiffness values by SWE, the pause between expiratory and inspiratory phase of respiratory cycle was used as the point of taking the readings. The mean of the 10 shear wave measurements at each anatomical window was recorded as the final SWE stiffness value in kPa (kilo Pascals). Unusual measurements were discarded.

These 4 anatomical windows were evaluated for performing shear wave elastography of liver in dogs on the basis of the consistency of results, ease of recording SWE and animal compliance. Consistency of results was recorded in terms of obtaining 10 consecutive measurements and was categorised as: good (8-10 consistent readings), fair (5-7 consistent readings), poor (2-4 consistent readings). Ease of recording SWE was categorised as: easy to scan (no anatomical hindrance in scanning the target organ), difficult to scan (scanning possible with adjustable hindrance like distended stomach or bowel, small intercostal space), impossible to scan (not possible to scan even after making adjustments). Animal compliance was recorded in terms of animal comfort and was categorized as: good (easy to record SWE measurements with no discomfort to the animal), satisfactory (animal allowed recording of SWE measurements with lot of reluctance), poor (animal did not allow recording of SWE measurements).

After selection of the ideal anatomical windows, for left and right liver lobes, the windows were evaluated for ideal recumbency (dorsal/lateral) on the basis of ease of acquiring 10 measurements and comfort of animal. Ease of acquiring SWE measurements was recorded in terms of obtaining 10 consecutive measurements in the respective recumbency and was categorised as: easy (8-10 consistent readings could be acquired), difficult (5-7 consistent readings could be acquired), and impossible (only 2-4 consistent readings could be acquired). Comfort of animal was recorded as either comfortable position for dog or uncomfortable position for recording the SWE measurements.

Comparisons of liver stiffness were made between the left and right liver lobes, different age groups, size and gender. The animals were divided on the basis of age group: less than 1 yr, 1-6 yr and more than 6 yr. The breed size was categorized as : large to giant breeds- Australian Shepherd dog, Chow Chow, Basset Hound, Siberian Husky, English Bulldog, Pit Bull Type Boxer, German shepherd dog, Golden Retriever, Labrador Retriever, American Bulldog, Medium sized breeds - Terrier, Pug, Boston Terrier, American Cocker Spaniel, Beagle and Non-descript and Small sized breeds- Shih Tzu, Pekingese, Dachshund, Bichon Frise, Rat Terrier, Jack Russell Terrier, Lhasa Apso, Miniature Schnauzer, Pomeranian) on the basis of body weight (Hawthorne *et al*., 2004). The mean±SE were calculated for various parameters. One way ANOVA was used to compare the groups for stiffness values among groups. Statistical analysis was done using SAS version 9.3 software.

Results and Discussion

To establish anatomical landmarks or windows for liver imaging 40 healthy dogs each were examined without sedation. Dogs presented for routine health check-up, vaccination, elective neutering with no abnormal clinical signs were subjected to haematological and biochemical evaluations. Nine different breeds included in the study *viz*., Labrador (12), Non-descript (12), German shepherd (4), Rottweiler (4), Golden retriever (2), Pug (2), Saint Bernard (1) and Beagle (1). twenty (50%) were male and 20 (50%) were female dogs. The distribution of age ranged from less than 1 yr (n=6), 1-6 yr (n=25), more than 6 yr (n=9) were recorded. The different breed sizes of dogs included in this study were small breed (n=5), medium size breed (n=12), and large size breed (n=23).

The haemato-biochemical parameters included for evaluation of liver were haemoglobin (Hb) total leukocyte count (TLC), packed cell volume (PCV), differential leukocyte count (DLC), platelets, aspartate aminotransferase (AST), alanine aminotransferase

(ALT) and alkaline phosphatase (ALP). The mean±SE value of Hb (12.07±0.35 g%), TLC (11.23±0.37 × 10^9/L), PCV (0.31±0.01 L/L), neutrophil (81.78±1.54 %), lymphocytes (15.1±1.29 %), monocytes (1.9±0.41 %), eosinophils (0.85±0.26 %), basophils (0.4±0.13 %), platelets (412±21.08 10^9/L) were recorded. Liver enzymes revealed an AST of 41.6±1.64 IU/L, ALT as 33.1±1.62 IU/L and ALP as 79.38±3.17 IU/L. The mean±SE values for all haemato-biochemical parameters were within normal reference range (James, 1999; Aiello *et al.*, 2016). The B-mode ultrasonography revealed uniform echotexture of liver parenchyma, which was hypoechoic to splenic parenchyma and isoechoic/hyperechoic to renal cortex. The hepatic parenchyma has a uniformly coarse echotexture, in which the larger blood vessels were visible (Fig. 1). Hepatic veins and portal veins were clearly remarkable (Godshalk *et al.*, 1988; Hartzband *et al.*, 1989).

After B-mode ultrasonography, shear wave elastography was performed using 1-5 MHz convex array transducer. Attempts were made to record SWE stiffness values (kPa) from four anatomical windows viz. left paracostal (LP), left 11th-12th intercostals (LICS), right paracostal (RP) and right 11th-12th intercostals (RICS). Region of interest (ROI) box was placed within 6-7 cm distance from Glisson's capsule in the liver (Fig. 2).

Fig. 1: Normal liver echotexture hypoechoic to splenic parenchyma or hyperechoic to renal cortex.

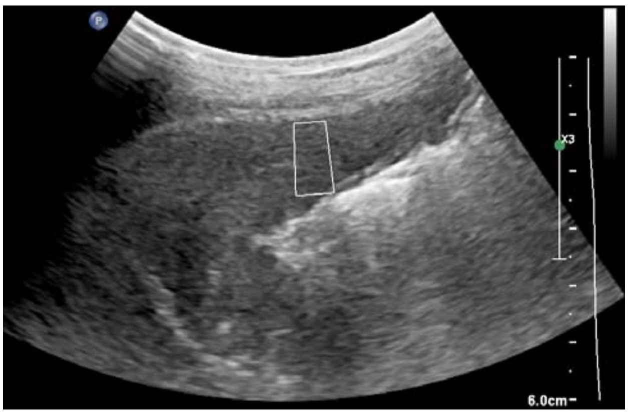

Fig. 2: Shear wave elastography (SWE) of left liver lobe.

For left liver lobes, left LP window was found to have good consistency for recording SWE in 36 dogs. In majority of these dogs 9-10 consistent measurements could be recorded while in a few dogs up to 8 consistent measurements were recorded. LP window showed fair consistency (5-7 consistent measurements) in 4 dogs while none of the dogs showed poor consistency. Similarly from LICS consistency of recording SWE was fair in 26 dogs and poor in 14 dogs. For the right liver lobes, RP window showed fair consistency in 2 dogs (5-7 consistent measurements) while 38 dogs were found to have poor consistency for recording SWE. Similarly from RICS all dogs fairly consistent results were obtained for recording SWE values (Fig. 3).

Ease of recording SWE values was evaluated for each window. It was easy to record SWE from LP window in all the 40 dogs, whereas LICS window was easy to record in only 28 dogs. For right liver lobes, RP window showed ease of recording to be difficult in 17 dogs and impossible in 23 dogs. Ease of recording from the RICS was easy in 37 dogs and impossible in 3 dogs. So from ease of recording, it can be concluded that left paracostal and right intercostal space had better liver lobe access and scanning as compared to left intercostal and right paracostal anatomical window (Fig. 4). Animal compliance was good in all the dogs for all anatomical windows. On

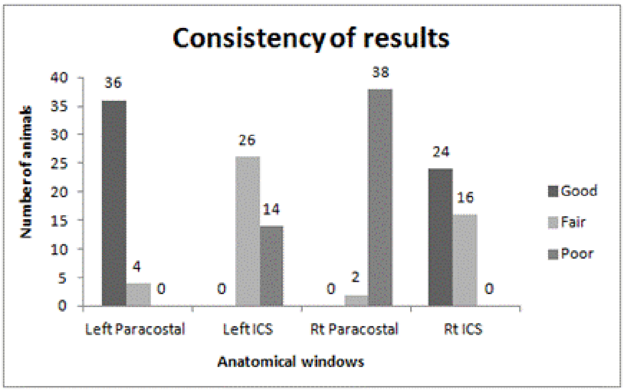

Fig. 3: Evaluation of different anatomical windows for performing shear wave elastography of liver in dogs on the basis of consistency of results.

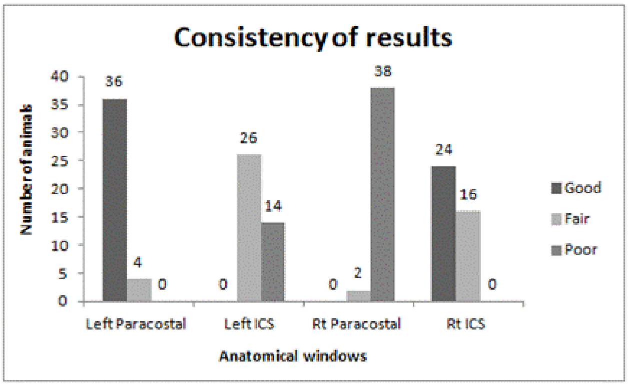

Fig. 4: Evaluation of different anatomical windows for performing shear wave elastography of liver in dogs on the basis of ease of scanning SWE.

the basis of above evaluation left paracostal for left liver lobe and right 11th-12th ICS for right liver lobe were selected as ideal windows for liver SWE. For left liver lobe, LP proved to be consistent and easy for recording SWE as compared to left ICS this can be due to smaller intercostal space as compare to no obstruction from paracostal window. For right liver lobes, right intercostals was better as compare to right paracostal, that could be attributed to more cranial position of right liver lobe. Based on above findings LP for left liver lobe in right lateral recumbency and right 11th-12th ICS for right liver lobe in left lateral recumbency were concluded as ideal windows for performing liver SWE. In humans also the subcostal approach has been recommended for obtaining good quality elastography image of middle and left segment of liver. For right liver lobe, humans have added advantage that overhead abduction of right arm can increase intercostal acoustic window from 9th-11th ICS (Barr *et al.*, 2015; Jeon *et al.*, 2015), which was not possible for dogs.

After selection of anatomical windows, the ideal recumbency (dorsal/lateral) was evaluated on the basis of ease of acquiring 10 consecutive measurements in an animal and comfort of the animal. For LP window the animals were positioned in right lateral and dorsal recumbency. Out of these two recumbencies right lateral was found to be easy for acquiring 10 measurements of liver in all the 40 dogs with no discomfort to the animal. Similarly for RICS window, left lateral recumbency was easy for acquiring measurements in all dogs through intercostal space without any discomfort to the dog. The dorsal recumbency was not comfortable for both the windows. Based on the above findings left paracostal for left liver lobe in right lateral recumbency and right 11th-12th ICS for right liver lobe in left lateral recumbency were concluded as ideal windows for performing shear wave elastography of liver in dogs.

The mean±SE value of the SWE measurements of healthy liver in 40 dogs was 2.09±0.11 kPa with the 95% confidence interval was 1.86-2.32 kPa for left lateral liver lobe and 2.23±0.13 kPa with the 95% confidence interval was 1.96-2.50 kPa for right medial lobe. There was no significant difference between left and right liver lobes (Table 1). The reported mean values for humans ranging from 3.43-4.24 kPa derived from 1.07-1.19 m/s shear wave velocity for Siemens Virtual touch quantification (VTQ) (Horster *et al.*, 2010; Popescu *et al.*, 2011; Son *et al.*, 2012), 3.49 kPa derived from 1.08 m/s shear wave velocity for Philips ElastPQ (Sporea *et al.*, 2014b) and 4.1-6 kPa for supersonic shear imaging (SSI) (Zoumpoulis *et al.*, 2011; Sirli *et al.*, 2013). In humans, by using transient elastography, liver stiffness values ranged 2.5-7 kPa. Fibrosis was likely to be mild or absent in normal liver, whereas for cirrhosis liver stiffness values were supposed to be higher than 12.5 kPa (Castera *et al.*, 2008). Another study reported that the reference range of normal hepatic elasticity was 2.6-6.2 kPa with 6.2 kPa as a cutoff value for human healthy liver (Chong *et al.*, 2014). The stiffness values for healthy dogs in the present study can be used as reference for evaluating cases suspected with liver diseases.

Table 1: Measurement of shear wave elastography values of liver in healthy dogs.

Site	No. of animals	Mean± SE(kPa)	95% Confidence Interval (CI)
Left paracostal	40	2.09±0.11	1.86-2.32
Right intercostal	40	2.23±0.13	1.96-2.5

For 20 males, mean±SE liver stiffness value for left lateral lobe was 2.09±0.67 kPa and 2.30±0.73 kPa for right medial lobe. Whereas for 20 females, mean±SE liver stiffness value for left lateral lobe was 2.09± 0.79 kPa and for right medial lobe was 2.16±0.95 kPa. The differences in SWE values among the male and female were non-significant (Table 2). In healthy dogs of less than 1 yr age, the mean±SE value of a liver stiffness was 1.1±0.09 kPa for left lateral lobe and 1.54±0.17 kPa for right medial lobe. For dogs of 1-6 yr, mean±SE value of a liver stiffness was 2.18±0.14 kPa for left lateral lobe and 2.26±0.17 kPa for right medial lobe. For patients of above 6 yr old, the mean±SE value of a liver stiffness was 2.49±0.16 kPa for left lateral lobe with and 2.6±0.26 kPa for right medial lobe. The mean±SE SWE of left lateral liver lobe of less than 1 yr old dog was significantly ($P \leq 0.01$) lower than the dogs belonging to other age groups (Table 3). This could be because of age related gradual changes in hepatic structure and function including hepatic sinusoidal endothelial cells (Le Couteur *et al.*, 2008). According to studies using ultrasound, the liver volume was found to be decreased by 20-40% for older as compared to younger persons (Wynne *et al.*, 1989; Le Couteur *et al.*, 1998). Multiple studies have addressed age as a variable of influencing liver stiffness in normal subjects, and the results have been inconsistent, reporting no difference across age groups (Ling *et al.*, 2013), but higher liver stiffness measurements (LSM's) was seen in older patients (Roulot *et al.*, 2011). In contrast to the present study, human studies have

Table 2: Measurement of shear wave elastography values of liver in male and female dogs.

Gender	Anatomical window	Mean± SE(kPa)	95% Confidence Interval (CI)
Male	Left paracostal	2.09±0.15	1.78-240
(n=20)	Right intercostal	2.29±0.16	1.95-2.64
Female	Left paracostal	2.09±0.18	1.71-2.46
(n=20)	Right intercostal	2.16±0.21	1.72-2.61

reported that age above 40 yr was associated with higher LSM (Bende *et al.*, 2018). Age has been attributed as one of the significant factors in fibrosis progression in chronic liver diseases (Lai *et al.*, 2003; Poynard *et al.*, 2003). However, due to short life span of dogs significant changes in stiffness of liver may not be appreciable in older dogs.

Table 3: Measurement of shear wave elastography values of liver in age groups less than 1 year, 1-6 years, more than 6 years dogs.

Age	Anatomical window	Mean± SE(kPa)	95% Confidence Interval (CI)
Less than	Left paracostal	1.1±0.09**	0.86-1.34
1 yr (n=6)	Right intercostal	1.54±0.17	1.1-1.98
1-6 yr	Left paracostal	2.18±0.14	1.9-2.46
(n=25)	Right intercostal	2.26±0.17	1.9-2.61
More than	Left paracostal	2.49±0.16	2.13-2.85
6 yr(n=9)	Right intercostal	2.60±0.26	2.0-3.2

**Differ significantly ($P \leq 0.01$) among various age groups for a particular window.

Similar to our study, ultrasonography and shear wave elastography performed on 127 healthy volunteers reported no significant difference in liver elasticity values between men and women (Arda *et al.*, 2013). In contrast, another study reported that in 2-D SWE; mean liver stiffness measurements (LSMs) were significantly higher for men than for women (Bende *et al.*, 2018; Colombo *et al.*, 2011).

In large breed dogs, the mean±SE value of liver stiffness was 2.11±0.15 kPa for left lateral lobe and 2.34±0.17 kPa for right medial lobe. For medium size breed dogs, the mean±SE SWE values was 1.86±0.21 kPa for left lateral lobe and 1.85±0.27 kPa for right medial lobe. The mean±SE SWE values for small breed dogs was 2.54±0.22 kPa for left lateral lobe and 2.6±0.25 kPa for right medial lobe. Liver stiffness values did not differ significantly among dogs belonging to different sizes (Table 4).

Table 4: Measurement of shear wave elastography values of liver in large, medium and small breed size dogs.

Size	Anatomical window	Mean± SE(kPa)	95% Confidence Interval (CI)
Large	Left paracostal	2.11±0.15	1.79-2.46
(n=23)	Right intercostal	2.34±0.17	2.0-2.69
Medium	Left paracostal	1.86±0.21	1.39-2.33
(n=12)	Right intercostal	1.85±0.27	1.26-2.44
Small	Left paracostal	2.54±0.22	1.93-2.15
(n=5)	Right intercostal	2.6±0.25	1.91-3.3

In conclusion it was found that left paracostal for left liver lobe in right lateral recumbency and right 11th-12th intercostal space for right liver lobe in left lateral recumbency were ideal windows for performing liver shear wave elastography in healthy dogs. The stiffness values of liver in healthy dogs range from 1.86-2.50 kPa.

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