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Quantification of Doppler Indices, Contrast Ultrasound Enhancement Phases and Perfusion Parameters of Hepatic Parenchyma in Healthy Dogs

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ABSTRACT

Background: The liver is a well-vascularized organ. Any pathology can impede its blood flow, making the liver the most suitable organ for studying Doppler indices and contrast-enhanced perfusion parameters.

Methods: This clinical study enrolled 9 healthy dogs including 2 breeds each of beagle, German shepherd and Labrador Retriever and one breed each of Crossbred, Pug and Pitbull. Spectral Doppler sonography and contrast-enhanced ultrasonography (CEUS) of liver vasculature using a second-generation contrast agent (Sonovue) were performed to quantify their normal pulsed-wave Doppler indices and contrast-enhanced perfusion parameters.

Result: The mean±SE values of Doppler indices of the portal vein were PSV (27.68±2.08 cm/s), EDV (15.32±1.74 cm/s), MV (14.05±1.94 cm/s), RI (0.44±0.05) and PI (0.93±0.11). Mean±SE value of Doppler indices of hepatic artery were 53.78±3.45 cm/s (PSV), 13.90±1.82 cm/s (EDV), 21.63±1.21 cm/s (MV), 0.73±0.04 (RI), 1.93±0.28 (PI) and for hepatic vein were 38.62±4.62 cm/s (PSV), 11.92±2.79 cm/s (EDV), 17.72±2.95 cm/s (MV), 0.69±0.06 (RI), 1.69±0.23 (PI). CEUS of hepatic parenchyma showed three phases viz arterial, portal and late portal phase. Mean±SE values of contrast-enhanced hepatic perfusion parameters (in seconds) were 7.22±0.40 (Arrival time), 16.33±1.38 (Time to initial peak), 30.22±1.29 (Time to final peak), 54.55±2.93 (Decline time) and 160.78±8.60 (Washout time).

Key words: Blood flow velocity, CEUS, Contrast-enhanced Perfusion phases, Hepatic vessels, Spectral Doppler indices.

INTRODUCTION

Ultrasonography plays a major role as a diagnostic imaging modality in assessing the morphology, echotexture and vascularity of the abdominal visceral organs (Cachard *et al*., 1997). However, B-mode ultrasonography is a less sensitive and specific modality because the pathology cannot be ruled out in some cases if a lesion is not observed and the ultrasonographic appearance of many lesions is found to be non-specific. To distinguish and further describe some lesions, Doppler and contrast-enhanced ultrasonography may be useful.

Doppler ultrasound offers both dynamic and anatomical information on the flow in the blood vessels supplying the visceral organs. Doppler ultrasonography relies on the Doppler shift principle, which involves a change in the frequency of sound waves when moving relative to the observer (Lamb, 2005). Doppler ultrasonography of the liver is primarily used to assess the flow direction, speed and spectral waveforms of the hepatic vessels (Moarabi *et al*., 2019). Changes in blood flow in the liver can be viewed as an early indicator of liver disease (Schneider *et al*., 1999). Doppler ultrasonography is helpful in the identification of numerous pathological conditions, such as portosystemic shunts (Carvalho *et al*., 2009), hepatic arterio-venous fistulas, cirrhosis of the liver, hepatomegaly, hemorrhage, congenital liver diseases, malignancies and abnormalities of the liver parenchyma (Ettinger and Feldman, 2005). Although Doppler ultrasonography provides information about blood flow, its use is limited in the imaging of microvasculature because of its low blood flow velocity and this limitation is overcome by the use of contrast agents. These contrast agents increase the accuracy of diagnostic ultrasound due to the virtue of their micro-vasculature filling ability (Shi *et al*., 2004). Furthermore, there is no radiation emission and it provides real-time images (Haers and Saunders, 2009).

Ultrasonographic contrast agents consist of tiny (1 to 7 µm in diameter), gas-filled micro-bubbles. These micro-bubbles being smaller than RBCs eliminate the risk of capillary embolism. Ultrasound waves get scattered by interacting with these contrast agents injected intravenously

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which results in the formation of a bright image of the vasculature.

Limited literature is available regarding the Doppler indices and CEUS perfusion parameters of the liver in clinically healthy dogs. Previous studies gave an overview of the Doppler and contrast perfusion parameters. Therefore, the present study was focused on providing detailed information on the quantification of these blood flow parameters to be used as reference values in canines.

MATERIALS AND METHODS

The present study was conducted on 9 healthy dogs (5 males and 4 females), aged 4.81±1.02 (9 months to 10 years) years and weighing 21.67±3.14 kg (7.5 to 35 kg), brought to Multi-Speciality Veterinary Hospital, GADVASU, Ludhiana, India, during the period of 2021 to 2023. Random breeds of dogs (2 breeds each of beagle, German shepherd and Labrador Retriever and one breed each of Crossbred, Pug and Pitbull) brought for routine check-ups to the veterinary hospital were included in the study after taking permission from the owners. IAEC permission vide number GADVASU/2021/IAEC/60/12 was taken for the use of contrast agents in these dogs. Inclusion criteria for healthy dogs were based on the absence of haemato-biochemical abnormalities, lack of visible parenchymal lesion on ultrasonography and absence of any concurrent abnormality at the time of examination. All the dogs were restrained on the ultrasound table without any sedation. B-mode and Colour Doppler examinations were performed in dorsal and lateral recumbency using Philips Affiniti 70G ultrasound machine to identify the hepatic vessels. These vessels were then subjected to spectral Doppler ultrasonography to demonstrate their Doppler indices. A sample volume gate between 1.5-2.0 mm in width and a Doppler angle of 60 degrees or less was used. Due to the smaller diameter of the hepatic artery, a narrowed sample volume gate (0.5-1.0 mm) was used. Clear waveform of the hepatic vessels (Hepatic artery, portal vein and hepatic vein) was captured and the ultrasound machine automatically calculated their respective spectral Doppler indices such as velocities (peak systolic velocity (PSV), end-diastolic velocity (EDV)), resistivity index (RI) and pulsatility index (PI). The formula for calculating average velocity, often known as mean velocity (MV), was;

$$MV = \frac{PSV\text{-}EDV}{PI}$$

The average of three measurements of these indices, ideally from separate vessel branches, was calculated.

Contrast-enhanced ultrasonography (CEUS) was then performed on all the dogs using a bolus injection of a second-generation ultrasound contrast agent Sonovue (Bracco Suisse SA, Geneva, Switzerland) comprising of sulphur hexafluoride gas-filled microbubbles encapsulated in a phospholipid shell @ 0.04 ml/kg body weight into the cephalic vein. This was immediately followed by a rapid bolus of 5 ml saline flush. Both the right intercostals approach (8-12th ICS) and sub-xiphoid approach using low frequency macro-convex transducer (1-5 MHz) was utilized to evaluate the liver by contrast ultrasonography. The whole scanning process was recorded for a period of 3-5 minutes and all the videos and pictures were periodically gathered in DICOM files. The interpretation was completed after comparing the images taken before and after the administration of contrast media. DICOM video was then analyzed using the contrast-specific software for quantification of contrast-enhanced perfusion parameters. A region of interest was selected in the hepatic parenchyma and motion compensation was selected to minimize the effects of movement during the analysis of the DICOM video. A graph was generated by the contrast software which was utilized to derive the contrast perfusion parameters *viz* arrival time *i.e*., the time to the first appearance of the contrast agent, time to peak (initial) *i.e*., the time when the intensity starts to rise following arrival time, time to peak (after) *i.e*., the time to peak intensity from the initial rise, decline time *i.e*., the time where the intensity starts declining and wash out time *i.e*., the time where the contrast agent starts to wash out from the organs were recorded. To quantify phases of hepatic contrast ultrasonography, 2 additional regions of interest were selected for the hepatic artery and portal vein at the porta hepatis through the right 8 to 12th intercostals space (Fig 1). The average of three measurements of these indices, ideally from separate vessel branches, was calculated. The quantitative data was represented as Mean±SE using Microsoft Excel.

RESULTS AND DISCUSSION

In the present study, the rate of blood flow to the liver and its related indices *viz* PSV, EDV, MV, PI and RI were evaluated in healthy dogs as shown in Table 1. These parameters are frequently used to detect vascular disorders in humans. The PSV value, in particular, has been established as one of the most dependable indicators in Doppler ultrasonography for detecting arterial stenosis (Scheinfeld *et al*., 2009). In the present study, the flow of the hepatic artery and portal vein (PV) was hepatopetal (toward the liver) and antegrade. The overall mean±SE values of Doppler indices of the portal vein were PSV (27.68±2.08 cm/s), EDV (15.32±1.74 cm/s), MV (14.05±1.94 cm/s), RI (0.44±0.05) and PI (0.93±0.11). Moarabi *et al*. (2019) reported similar values of RI (0.48±0.10) and PI (0.72±0.26) in healthy cats. According to Popov *et al*. (2012), the PSV of the portal vein in humans was 28.68±6.12 cm/s, which was consistent with the results of the current study in dogs. Similar values of the mean velocity values of the portal vein (14.70±2.50 cm/s and 17.39±4.77 cm/s) were reported by Finn-Bodner and Hudson (1998) and Sartor *et al*. (2010) respectively.

Mean±SE value of Doppler indices of hepatic artery were 53.78±3.45 cm/s (PSV), 13.90±1.82 cm/s (EDV), 21.63±1.21 cm/s (MV), 0.73±0.04 (RI), 1.93±0.28 (PI) and for hepatic vein were 38.62±4.62 cm/s (PSV), 11.92±2.79

cm/s (EDV), 17.72±2.95 cm/s (MV), 0.69±0.06 (RI), 1.69±0.23 (PI). Hepatic artery Doppler indices have been reported earlier for a variety of species, including humans (PSV: 69.60±20.55 cm/s; EDV: 23.41±7.13 cm/s; PI: 1.06±0.12) (Popov *et al*., 2012), cats (PSV: 49.79±9.45 cm/s; EDV: 31.92±5.05 cm/s; PI: 0.85±0.20) (Morabi *et al*., 2019), rabbits (PSV: 34.12±3.24 cm/s, PI: 0.87±0.11) (Maher *et al*., 2020). Smithenson *et al*. (2004) carried out studies on hepatic vein Doppler indices in healthy anesthetized dogs and reported a PSV (S-wave) value of 20.10±10.50 cm/s and EDV (D-wave) value of 14.30±6.90 cm/s in a hyperdynamic state. The fundamental reason for the substantially higher PSV values for the hepatic vein in the current investigation may be related to the breathing changes that may have raised the forward flow below the baseline, resulting in a taller S-wave (Szatmari *et al*., 2001).

Doppler indices of the hepatic vein are clinically important since these indices get changed on both hepatic and cardiac affections (Scheinfeld *et al*., 2009). Moreover, the indices of the portal and hepatic vein can provide useful information to detect congenital or acquired vascular affections of the liver (Nyland and Mattoon, 2002). Schneider *et al*. (1999) measured the PI value of liver arteries and the velocity of venous blood flow in patients with cirrhosis and concluded that PI increased significantly in comparison to the control healthy group. In the present study, hepatic artery Doppler indices were quantified and these indices can be used to predict hepatic diseases as the values of indices get increased.

Liver on CEUS examination showed three phases namely the arterial phase, portal venous phase and late portal phase. The arterial phase started as soon as the intrahepatic artery showed mild enhancement. This shows the arrival of a contrast agent in the liver. This mild enhancement of the hepatic artery is then followed by marked enhancement of the hepatic artery and a mild enhancement of the parenchyma (initial parenchymal peak phase). The portal venous phase started with the mild enhancement of the portal vein. This mild enhancement of the portal vein is followed by the final peak phase wherein

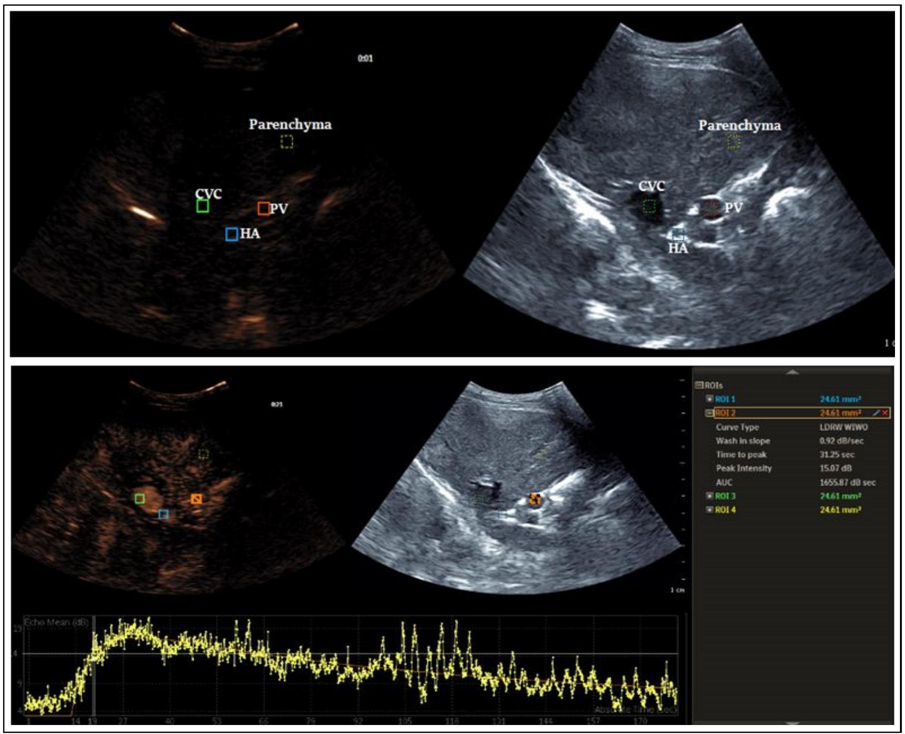


Fig 1: Placement of region of interest in the portal vein (PV), hepatic artery (HA) and hepatic parenchyma for quantification of contrast-enhanced perfusion indices.

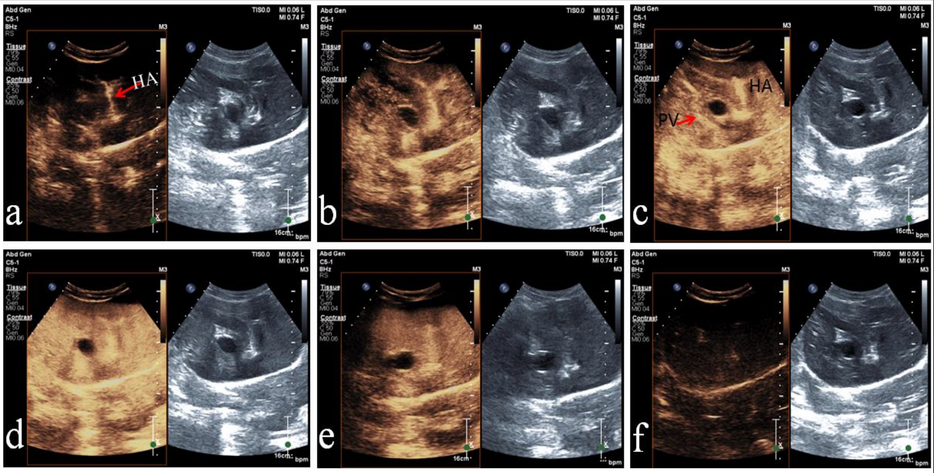
Table 1: Mean±SE value of Spectral Doppler indices of hepatic vessels in healthy dogs.

Vessels	PSV (cm/s)	EDV (cm/s)	MV (cm/s)	RI	PI
Portal vein	27.69±2.08	15.32±1.74	14.06±1.94	0.43±0.05	0.93±0.11
Hepatic artery	53.78±3.45	13.90±1.82	21.63±1.21	0.73±0.04	1.93±0.28
Hepatic vein	38.62±4.62	11.92±2.79	17.72±2.95	0.69±0.06	1.69±0.23

PSV- Peak systolic velocity; EDV- End diastolic velocity; MV- Mean velocity; RI- Resistivity index; PI- Pulsatility index.

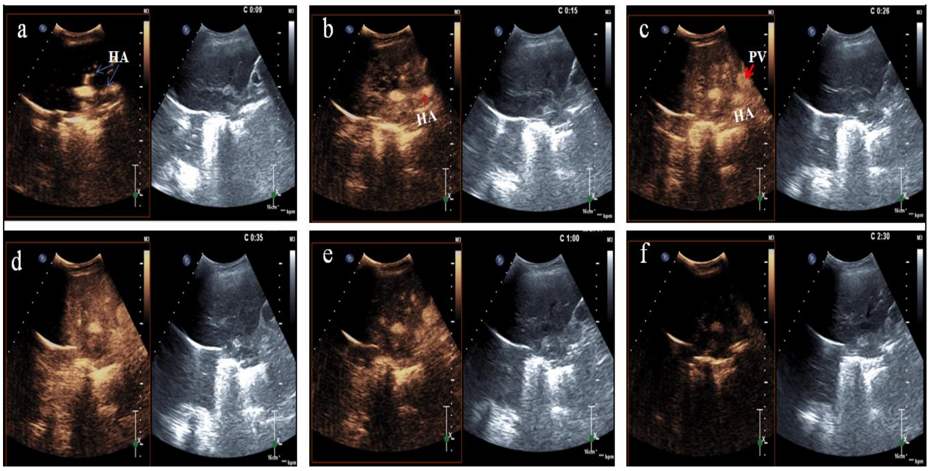
the entire liver parenchyma was markedly enhanced. The third phase is the late phase (Declining phase to washout phase) wherein the hepatic parenchyma showed a decline of enhancement from the hepatic parenchyma (Declining phase) and at the end of this phase liver became non-enhanced (Washout phase). The enhancement phases of hepatic CEUS examination *via* the sub-xiphoid approach and right lateral intercostals approach are shown in Fig 2 and Fig 3 respectively. After the subjective analysis, regions of interest were selected for the hepatic artery, portal vein and hepatic parenchyma for objective analysis (Fig 1).

The mean±SE of the contrast perfusion parameters (in seconds) of the liver is shown in Table 2. During the CEUS examination of the liver, the hepatic arterial enhancement (arterial phase) started at 7.22±0.40 seconds and this enhancement lasted up to 29.56±0.73 seconds. The portal vein enhancement (portal venous phase) followed the arterial enhancement. The portal phase



a) Arterial phase arrival time, b) Marked enhancement of hepatic artery and mild enhancement of hepatic parenchyma, c) Portal phase characterized by enhancement of portal vein (arrow), d) Peak portal phase characterized by marked homogenous enhancement of entire hepatic parenchyma, e) Late phase characterized by declining of parenchymal enhancement and f) Washout phase characterized by loss of enhancement of entire parenchyma.

Fig 2: CEUS of the liver through sub-xiphoid approach.



a) Arterial phase arrival time, b) Marked enhancement of hepatic artery (arrow) and mild enhancement of hepatic parenchyma, c) Portal phase characterized by enhancement of portal vein (arrow), d) Peak portal phase characterized by marked homogenous enhancement of entire hepatic parenchyma, e) Late phase characterized by declining of parenchymal enhancement and f) Washout phase characterized by loss of enhancement of entire parenchyma.

Fig 3: CEUS of the liver through right lateral intercostal approach.

Table 2: Mean±SE value of contrast perfusion parameters (in seconds) in different phases of hepatic contrast ultrasonography.

Arterial phase		Portal phase		Late phase	
Arrival phase or HAE_i	Initial parenchymal peak phase or HAE_p	PVE_i phase	Final parenchymal peak phase or PVE_p phase	Decline phase	Washout phase
7.22±0.40 sec	16.33±1.38 sec	27.89±0.54 sec	30.22±1.29 sec	54.55±2.93 sec	160.78±8.60 sec

HAE_i- Hepatic artery initial enhancement; HAE_p- Hepatic artery peak enhancement; PVE_i- Portal vein initial enhancement; PVE_p- Portal vein peak enhancement; Sec- Seconds.

enhancement began at 27.89±0.54 seconds and lasts up to 54.55±2.93 seconds.

After the portal phase, the decline phase began wherein the parenchymal enhancement starts to fall down and it took 160.78±8.60 seconds for the parenchymal enhancement to return to baseline as the contrast medium left the liver by the caudal vena cava.

Nyman *et al*. (2005) reported similar phases during the CEUS examination of the normal canine liver. They reported the arterial phase to start at 7-10 seconds after the administration of the contrast agent with a duration of 10-15 seconds. This was followed by the portal venous phase at 30-45 seconds which lasted up to 150-200 seconds.

Since contrast agents have the potential to fill the microvasculature, CEUS can detect changes in tissue perfusion even in the smallest branches. CEUS can identify intra-tumoral vascular supply and hence can accurately characterize focal liver lesions (Wilson and Burns, 2006). CEUS has been used in humans to diagnose portal hypertension in a non-invasive manner via measurement of contrast agent arrival time in the hepatic veins (HVAT), which is inversely related to the severity of the liver disease. Decreased HVAT shows that the severity of liver disease has risen (Kim *et al*., 2012). The hepatic vein transit time (HVTT) can also be used to distinguish between hepatitis and cirrhosis, especially in situations when the biopsy is contraindicated (Lim *et al*., 2005).

CEUS can also diagnose hepatic trauma. During the portal, venous and late phases of CEUS, the normal liver is uniformly enhanced. The traumatic region (lacerations or contusions) is devoid of the vascular supply and exhibits absent or diminished perfusion, which is noticeable as a hypo-enhanced area (Cagini *et al*., 2013). CEUS increases the sensitivity, specificity, positive predictive value and negative predictive value of B-mode ultrasonography for diagnosing solid organ damage (Valentino *et al*., 2006).

CONCLUSION

Doppler indices and perfusion parameters must be standardized to measure blood flow to the liver. The present study does preliminary work and forms a reference base for further standardization which requires the involvement of a large sample size. These standard values would allow the sonographer to distinguish between affected and healthy parenchyma as any variations in hepatic Doppler indices and perfusion parameters could indicate a diseased state.

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Conflict of interest: None.

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