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SHORT COMMUNICATION

Reference values and repeatability of pulsed-wave Doppler flowmetry of portal vein in healthy dromedary she-camels

Ahmed A. Elsayed[1], Naglaa A. Gomaa[2]
[1]Department of Animal Health and Poultry, Animal and Poultry Production Division, Desert Research Center DRC, Matariya, Egypt
[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El Sheikh, Egypt

ABSTRACT

Objective: This study was conducted to establish the normal values and repeatability of pulsed-wave (PW) Doppler ultrasonographic parameters of the hepatic portal circulation in healthy dromedary she-camels.

Materials and Methods: According to the American Society of Veterinary Clinical Pathology guidelines, the sample size and statistical analysis were followed. Ten healthy, non-pregnant, non-lactating she-camels were selected after physical, hematological, and ultrasonographic examinations. All hepatic ultrasonographic measurements were obtained from unsedated standing she-camels at the 11th right intercostal space using B-mode and PW Doppler.

Results: The ultrasonographic measurements were portal vein (PV) diameter 1.76 ± 0.37 cm; portal velocity 12.3 ± 3.2 cm/sec; portal area 2.05 ± 0.57 $(cm)^2$; portal congestion index 0.16 ± 0.04 cms; and portal blood flow volume 0.02 ± 0.05 ml/sec/kg. The intra-assay coefficient of variations (CV)% of the above-mentioned variables were 5.84 ± 4.32, 12.3 ± 7.1, 10.5 ± 5.8, 14.7 ± 7.1, and 15.8 ± 12, respectively. However, the inter-assay CV% were 13.2 ± 5.9, 18.5 ± 8.4, 19.7 ± 7.6, 17.3 ± 8.8, and 31.9 ± 15, respectively.

Conclusion: This study provides data that may be used as reference values for Doppler measurements of the PV in she-camels, which may help diagnose some hepatic disorders in camels.

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KEYWORDS

Portal vein; PW Doppler; reference values; repeatability; she-camels.

Introduction

Doppler ultrasound is a practical, non-invasive imaging diagnostic technique that has been used to characterize the blood flow associated with normal and pathological conditions in veterinary medicine [1]. Hepatic diseases are relatively common in camels [2], but many of them are not diagnosed because the signs are nonspecific. Hematology and serum biochemistry are not enough sometimes to clarify the nature of the disease [3]. Routine hepatic ultrasonography is a reasonably sensitive method for detecting localized lesions. However, it may be unsatisfactory for the diagnosis of diffuse lesions due to the overlapping of sonographic signals [4]. Diffuse hepatic diseases often lead to abnormalities in hepatic circulation identified by Doppler ultrasonography [5]. This is considered an excellent method for the diagnosis of chronic and diffuse hepatic diseases. Doppler ultrasonography is also useful in predicting and determining the severity of chronic and diffuse hepatic disorders [6,7].

Portal vein (PV) is the main vessel with a high percentage of total blood supply of hepatic circulation, so it is the most affected vessel by hepatic lesions [8]. The establishment of the reference values for portal flow parameters using pulsed-wave (PW) Doppler is essential in the interpretation and diagnosis of many hepatic diseases [9]. The reference values for PW Doppler measurements of the PV in she-camels are still lacking. Hence, the present study was delineated to provide reference values for some PW Doppler parameters of the PV in she-camels. We hypothesized that such data could be helpful for the diagnosis of hepatic diseases in camels.

Correspondence Naglaa A. Gomaa ✉ naglaagomaa505@yahoo.com Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

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Materials and Methods

Ethical approval

The Research Ethical Committee of the Faculty of Veterinary Medicine, Kafrelshiekh University, Egypt, approved all experimental procedures [Approval No. KFS-IACUC/18/6/2019].

Animals

A total of 10 healthy, non-pregnant, non-lactating dromedary she-camels were used in this study. This was considered an acceptable number for measuring reference values in veterinary species, according to the American Society of Veterinary Clinical Pathology (ASVCP) guidelines. The camels' bodyweight ranged from 387 to 634 kg, and age ranged from 18 to 22 years. They belonged to the Mariut Research Station, Desert Research Center, El-Amria, Alexandria, Egypt. The camels were considered clinically healthy based on physical examination, hematological, and serum biochemical examinations [10]. Camels were housed in an open yard and fed a maintenance ration composed of a concentrated mixture and Egyptian clover hay (*Trifolium alexandrinum*) and fresh water *ad libitum*.

Ultrasonographic examination

Doppler ultrasonography was adopted using a real-time B-mode ultrasound machine (Samsung Medison SONOACE R3 ultrasound system, South Korea) and a dual convex transducer (2–8 MHz). Camels were examined in stocks in standing position without sedation. The convex transducer was placed at the 10th or 11th intercostal space (ICS) in which the porta hepatis could be observed [11]. The portal vein diameter (PVD) was measured at a cross-sectional plane using ultrasonographic cursors. After that, the cross-sectional PV areas were calculated from the following formula:

Area of PV = (Diameter of the portal vein)^2 × 3.14/4 [8].

The transducer was kept at the porta hepatis and then moved approximately one ICS cranially to reach the PV's right branch [11]. Color Doppler was carried out on the PV's right branch for subjective evaluation of the portal blood flow direction and velocity. After that, it was switched to PW Doppler for the quantitative determination of the PV velocity. The Doppler gate was 9 mm and was placed inside the vessel. The insonation angle between the Doppler beam and the PV's long axis' right branch was less than 60° in all examined camels for accurate calculation of portal velocities [12]. When the image did not show any aliasing, the mean velocity (V_{mean}) was measured manually by measuring three nonconsecutive waves. Based on the obtained data, portal congestion index (CI) and average portal blood flow volume (PBFV) were assessed according to the following formulas:

$$CI\ (cms) = A\ (cms)^2 / V_{mean}\ (cm/sec)$$

where CI is the CI; A is the PV area; and V_{mean} is the PV flow mean velocity.

$$PBFV = V_{mean}\ (cm/m) \times A\ (cms)^2 / BW(kg)$$

where PBFV is the Portal Blood Flow Volume and BW is the body weight [5,13].

Statistical analysis

According to the ASVCP guidelines, the sample size and statistical procedures were followed. For estimating the repeatability, Doppler ultrasonographic examination was carried out 3 days within a 1-week interval by the same observer. On the same day, each parameter of PW Doppler was measured three times. The repeatability of the PW Doppler ultrasonography was assessed by calculating the intra-assay and inter-assay coefficient of variations (CV). The degree of variability of each parameter was as follows: CV% lower than 10% was considered as low variability; CV% within 10%–20% was considered as moderate variability, and CV% higher than 20% was considered as high variability [14]. Spearman's correlation coefficient was calculated to emphasize the correlation between the measured parameters and body weight [15]. All statistical procedures were carried out with a commercially available software program (Graph Pad Prism, San Diego, CA). $p < 0.05$ was considered as significant.

Results

In all the examined camels, the liver was visible by ultrasound between the right caudal flank and the right 9th ICS. All investigated camels did not have any B-mode ultrasonographic abnormalities in the liver or adjacent structures. As a guide to visualize the PV, the characteristic typical identifiable inverted T-shape of splenic and cranial mesenteric veins is shown in Figure 1a. The color Doppler examination of the PV showed an excellent velocity with a one-way blood flow direction, as shown in Figure 1b. The ultrasonographic measurements were PV diameter and the PV (V_{mean}), as shown in Figure 1c and d. The summary statistics (mean ± SD, range, and percentiles) for PV are presented in Table 1. The frequency distribution of hepatic portal hemodynamics measurements are listed in Table 2. The mean ± SD of intra-assay CV for PV ultrasonographic parameters are presented in Table 3. On the contrary, the inter-assay CV% for the same variables are listed in Table 4. Our findings showed a non-significant variation among intra-assay CV% at different days and inter-assay CV% at

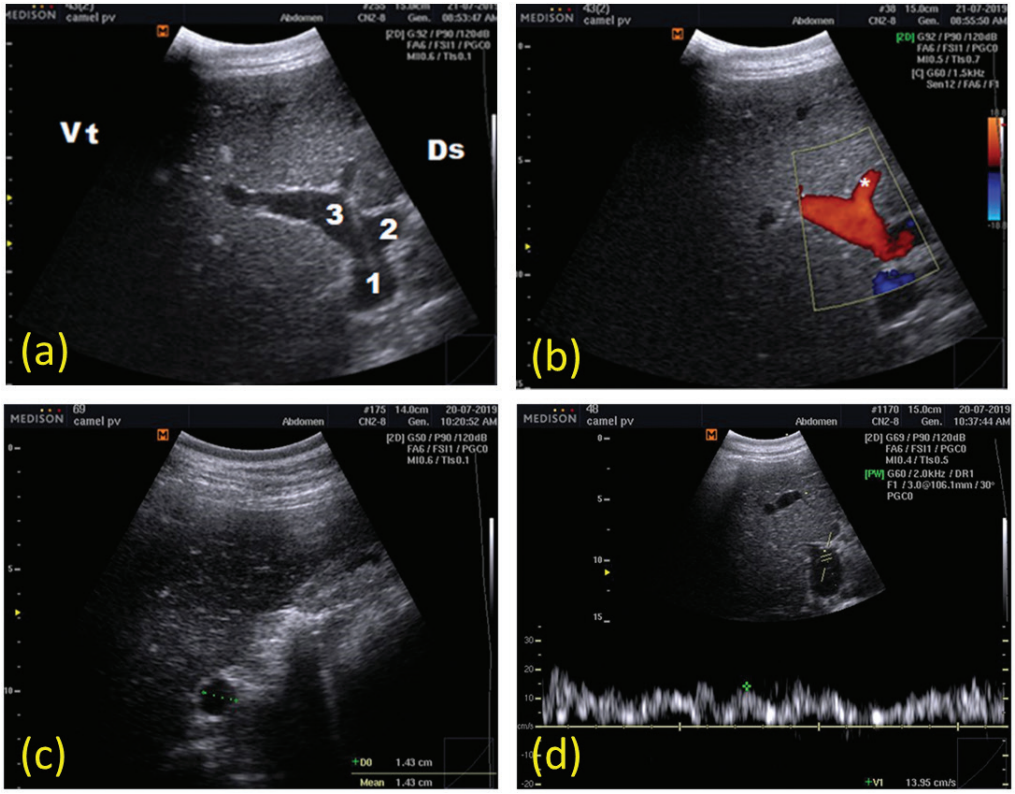

Figure 1. Ultrasound and color Doppler images of hepatic conditions. (a) Ultrasound image showing the typical inverted "T" shape of the porta hepatis at the 11th ICS. 1. Splenic vein; 2. mesenteric vein; 3. PV. Ds = dorsal; Vt = ventral. (b) Color Doppler mapping showing the origin of the PV's right branch in camel (*). The longitudinal section at approximately the right 11th ICS. (c) B-mode ultrasound image of the transverse section of the PV shows its diameter at the 11th ICS. (d) PW Doppler of the right branch of the PV of a camel. The longitudinal section at approximately the right 11th ICS. Note the insonation angle (30°).

Table 1. Summary statistics of PW Doppler ultrasonographic measurements of the hepatic PV in healthy dromedary she-camels (*n* = 10).

Variables	Percentile				Median (Range)	95% CI	Mean ± SD
	90%	75%	25%	10%			
PV diameter (cm)	2.3	1.94	1.47	1.33	1.72 (1.12–2.84)	1.68–1.84	1.76 ± 0.37
PV velocity (cm/sec)	16.83	14.66	9.45	8.67	11.98 (6.35–21.8)	11.5–13	12.3 ± 3.2
PV area $(cm)^2$	2.89	2.48	1.65	1.2	2.2 (1–3.14)	1.93–2.18	2.05 ± 0.57
PV CI (cm sec)	0.21	0.19	0.12	0.1	0.16 (0.07–0.24)	0.151–0.169	0.16 ± 0.04
PV BFV (ml/sec/kg)	0.08	0.06	0.03	0.02	0.05 (0.01–0.12)	0.04–0.05	0.02 ± 0.05

PV = Portal vein; CI = Congestion index; BFV = Blood flow volume.

other reads per day for Doppler ultrasonographic measurements except Portal Vein Blood Flow Volume (PVBFV), which showed a higher variation.

Discussion

Although liver biopsy is considered as the best standard for accurate diagnosis of most hepatic diseases, Doppler ultrasonography can be an excellent tool for diagnosing hepatopathies associated mainly with vascular changes and chronic diffuse hepatic diseases [16]. In the present study, the liver was visible by ultrasound between the right caudal flank and the right 9th ICS, as reported in a previous study [17]. All investigated camels did not have any ultrasonographic abnormalities in the liver or adjacent structures by the B-mode examination. The PV could be distinguished

Table 2. Frequency distribution of PW Doppler ultrasonographic measurements of the hepatic PV in healthy dromedary she-camels (*n* = 10).

Variables	Lowest frequency		Highest frequency	
	(%)	Value	(%)	Value
PV diameter (cm)	5 (6%)	≥ 2.6–3	52 (64.1 %)	≥ 1.4–1.8
PV velocity (cm/sec)	7(8.6%)	≥ 18–22	63 (77.7%)	≥ 10–16
PV area $(cm)^2$	2(2.4%)	≥ 0.8–1	31 (38.2%)	≥ 2–2.4
PV CI (cm sec)	1(1.2%)	≥ 0.06–0.08	39 (48.1%)	≥ 16–20
PV BFV (ml/sec/kg)	2(2.4%)	≥ 0.11–0.13	50 (70.3%)	≥ 0.3–0.6

Ten camels were examined via Doppler ultrasonography for 3 days with a 1-week interval. On each day, three PW Doppler ultrasonography measurements with a variance <5% were recorded. Thus, by the end of the study, there were nine PW Doppler ultrasonography measurements for each camel with a total of 90 measurements.
PV = Portal vein; CI = Congestion index; BFV = Blood flow volume.

Table 3. Intra-assay CVs of PW Doppler ultrasonographic measurements of the hepatic PV in healthy dromedary she-camels (*n* = 10).

Variables	Percentile				Median (Range)	95% CI	Mean ± SD
	90%	75%	25%	10%			
PV diameter (cm)	11.4	6.6	3	2	5 (1.5–22.2)	4.1–7.5	5.84 ± 4.32
PV velocity (cm/sec)	22.8	16	6.2	3.5	11.2 (2–28)	9.4–15.1	12.3 ± 7.1
PV area $(cm)^2$	21.1	14.2	5.9	3.3	10.2 (2.6–22.3)	8.2–12.8	10.5 ± 5.8
PV CI (cm sec)	25.1	20.3	9	5	15.7 (2.3–27.7)	11.9–17.6	14.7 ± 7.1
PV BFV (ml/sec/kg)	33.5	24.7	6.1	1	15.7 (1–43.3)	11–20.6	15.8 ± 12

PV = Portal vein; CI = Congestion index; BFV = Blood flow volume.

Table 4. Inter-assay CVs of PW Doppler ultrasonographic measurements of the hepatic PV in healthy dromedary she-camels (*n* = 10).

Variables	Percentile				Median (Range)	95% CI	Mean ± SD
	90%	75%	25%	10%			
PV diameter (cm)	23.6	17.3	8.7	6	12.6 (2.7–25.9)	10.8–15.5	13.2 ± 5.9
PV velocity (cm/sec)	30.5	23.9	12.7	6.5	19.3 (2.8–32)	15.1–21.8	18.5 ± 8.4
PV area $(cm)^2$	28	25.9	12.7	8.8	18.5 (5.2–29.6)	16–22	1 9.7 ± 7.6
PV CI (cm sec)	29	25.7	9.3	4.4	19.2 (2.8–33.6)	13.8–20.8	17.3 ± 8.8
PV BFV (ml/sec/kg)	56.5	40.1	22.5	13.1	26.9 (10.1–80.9)	25.6–38.1	31.9 ± 15.7

PV = Portal vein; CI = Congestion index; BFV = Blood flow volume.

from other hepatic blood vessels by its hyperechoic wall. It is located ventrally and laterally to the caudal vena cava. Its characteristic radiating pattern of the hepatic parenchyma and circular appearance on cross-sectional view are also distinguishable features, as supported previously [18].

PV diameter was easily measured in the 11th ICS of each camel utilizing B-mode. However, a previous study reported great accessibility of PV from the 9th to 11th ICS in camels positioned in sternal recumbency [17]. The PV diameter in the present research at the 11th ICS was less than that recorded in healthy camels examined at sternal recumbency (1.76 ± 0.37 *vs.* 3.3 ± 0.5 cm) [17]. This could be attributed to the difference in age, weight, and posture of the investigated animals. It is essential to measure PVD to calculate portal blood flow necessary for cirrhosis diagnosis [19]. The mean velocity of PV is usually measured from the right branch as the insonation angle may be at its minimal degree, making its use appropriate for the measurement of flow velocity [20]. In our work, the mean PV velocity was 12.3 ± 3.2 (cm/sec), with a range of 6.35–21.8 cm/sec. It has been reported that the mean velocity of portal blood flow decreased in dogs with chronic hepatic

diseases, portal hypertension, and cirrhosis [8,7]. There was a non-significant correlation (p = 0.647) between the animals' PV mean velocity and body weight in this study. This result coincided with that obtained in dogs [21]. The area of PV in the examined healthy camels was 2.05 ± 0.57 $(cm)^2$, with a range of 1–3.14 $(cm)^2$. The current work showed a non-significant correlation (p = 0.724) between the animals' PV area and body weight. On the contrary, it was found that the PV area in small-sized dogs is significantly smaller than that of larger ones [21].

The measurement of PBFV has significant importance in evaluating hepatic circulation since the PV is carrying, on average, 75% of the total blood received by the liver [4]. In this study, we found a negative correlation between PVBFV and the animal's body weight (r = −0.717; p = 0.029). This finding is in agreement with a previous study [16].

The measurement of Portal Vein Congestive Index (PVCI) is an excellent indicator of chronic hepatic disorders [6]. In the present study, the PVCI was 0.16 ± 0.04 cm/sec in healthy camels. The reduction of PVCI was observed in patients with chronic liver disease, cirrhosis, and portal hypertension [13]. This study showed a non-significant correlation (p = 0.938) between PVCI and the animals' body weight. However, another study reported that the PVCI in dogs is significantly associated with bodyweight [21].

In our investigations, we calculated the CV% to assess the precision and the repeatability of the PW Doppler ultrasonographic measurements of the hepatic PV. The mean intra-assay and inter-assay CV% and the associated 95% CI of PV diameter, PV area, and PV mean velocity. PVC was within a moderate variability (10%–20%) except the PVBFV, which showed a higher variability (20%), especially inter-assay CV, which could be attributed to the wide range of bodyweight of the examined camels [14]. These results matched the previous study on dogs [21], where it was reported that the Doppler ultrasonographic measurements of the PVBFV were affected by body weight.

The present study's limitations were the small number of examined camels that were only female with a narrow age range and a wide range of body weight that significantly affected PVBFV. Therefore, further investigations are needed to test the impact of age, sex, and other physiological factors on such variables.

Conclusion

It can be concluded that the present study shows good repeatability or acceptable intra- and inter-assay variability for all ultrasonographic measurements, except for PVBFV, as it is influenced by body weight. The study provides useful data that can be used as reference values during Doppler ultrasonographic examination of portal blood flow in dromedary she-camels, which could help diagnose some of their hepatic disorders.

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Conflict of interest

The authors declare no conflicts of interest.

Authors' contribution

AAE designed the study and made the statistical examination and interpretation of data. NAG made the ultrasonographic examinations involved in drafting and revision of the manuscript and its preparation for submission. All authors read and approved the final manuscript.

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