Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Ultrasound image analysis for detection of fatty liver and grading its severity in buffaloes at Mosul, Iraq

Z.H. Hasan[1], O.M. Al-Iraqi[1] and I.O. Abdul Majjed[2]

[1]Department of Medicine, College of Veterinary Medicine, [2]Department of Computer Sciences, College of Computer Sciences and Mathematics, University of Mosul, Mosul, Iraq

Article information

Article history:
Received June 29, 2021
Accepted September 3, 2021
Available online March 13, 2022

Keywords:
Buffaloes
Fatty liver
Ultrasound analysis
β-hydroxybutyrate

Correspondence:
O.M. Al-Iraqi
osamamuwafaq@uomosul.edu.iq

Abstract

The study aimed to (i) examines whether quantitative and qualitative digital analysis of hepatic ultrasound images can be used to detect fatty liver and grading its severity, (ii) to estimate the concentration of serum β-hydroxybutyrate content in normal and diseased animals. A total of 50 buffaloes (15 clinically normal and 35 suffered from decreased milk production, loss of appetite, and loss of body weight) 2-4 weeks after parturition were studied. Buffaloes were examined by real-time ultrasound using a 3.5 MHz convex probe. Ultrasound images were saved for qualitative and quantitative analysis, using computer aid Fatty-Liver software (programmed in Matlab language). Based on the digital analysis of ultrasound images, the diseased buffaloes were classified as mild (n=20), moderate (n=7) and severe (n=8) fatty liver affection. Results indicated that the concentration of β-hydroxybutyrate estimated via ELISA were 0.409, 0.735, 0.923, 1.708 mmol/dL in normal, mild, moderate, and severe diseased buffaloes respectively. However, a significant difference has been encountered among normal and diseased animals. It has been concluded that buffaloes at Mosul suffered from fatty liver and digital analysis of ultrasound images was a suitable method for clinical diagnosis, furthermore, estimation of β-hydroxybutyrate was a suitable method for laboratory diagnosis.

DOI: 10.33899/ijvs.2021.130666.1864, ©Authors, 2022, College of Veterinary Medicine, University of Mosul.

Introduction

Fatty liver is a metabolic disease that affect ruminants and occur when the animal suffered from negative energy balance mainly after parturition , however, about 50% of cow affected at 4 week postpartum resulting in losses in about 60 million US dollar (1). The disease cause interference with animal production, fertility and performance (2). Cattle commonly affected with fatty liver because of having less ability to export the accumulated fat in their hepatocyte related to decrease hepatic activity and thus resulted in an increase of the non-esterified fatty acid, acetoacetate, and β-hydroxybutyrate (3). Fasciolosis considered as important cause of liver damage (4,5) which could predispose to fatty liver syndrome (6). Fatty liver is defined as the percentage of liver lipid or Triacylglycerol (TGA), which is associated with disrupted well-being, health status and reproductive performance of dairy cows and early culling of diseased animals. Fatty liver causes many defects in liver parenchyma such as fatty cysts, increased hepatocytes size, damage of mitochondria, nuclei volume compression and the decrease of organelles. The severity of Fatty liver depends on the accumulation extension of TGA and can be classified to mild, moderate, and severe (2).

The diagnosis of fatty liver depends on the triacylglycerol concentration by using biopsy and other laboratory examination methods, including the biochemical analysis, however, it has been shown that, ELISA are more useful for detecting β-hydroxybutyrate (BHB) and Non-esterified fatty acids (NEFA) (7,8). The liver consists of fibrofatty tissues, which give parallel echogenicities by ultrasound (9). Ultrasound as a non-invasive technique, which was recently used for clinical detection of many diseases like left displaced abomasum (LDA) (10), fatty abscess (11), fatty liver in camels (12), dairy cattle and buffaloes (6,13,14). The real-time images of the liver give a benefit of ultrasound for showing actual hepatic parenchymal changes (15). Digital analysis has the highest sensitivity, specificity, accuracy and positive and negative predictive values for diagnosing the fatty liver followed by ultrasonography (16). The quantitative analysis characterized by increase echogenicity of liver parenchyma, deep attenuation and vascular blurring (15), while qualitative analysis relies on the computer-aided digital analysis which represents the future of ultrasound (17,18). Moreover, recently, there has been a new quantification method of liver fat, conducted by using Magnetic Resonance Imaging (19). However, the buffaloes in Mosul suffer from metabolic disorders (20) and some of them have low reproductive performance (21,22) hence, the current study was conducted to detect the fatty liver and grading its severity in buffaloes using quantitative image analysis, and to evaluate the serum β-hydroxybutyrate concentration using ELISA.

Materials and methods

Animals

The current study was conducted to examine 50 female buffaloes 4-12 years and 2-4 weeks after calving from different areas of Mosul governorate, Iraq, form the period started in September 2020 to February 2021. Thirty-five buffaloes show signs of loss of appetite and decrease in both of milk production and body weight loss were selected. Furthermore, 15 clinically normal buffaloes were selected as the control group.

Ultrasonographic examination

The liver was scanned by using a KX5100VET real time ultrasound portable machine at the standing position after controlling the animal without using anesthesia. Machine setting was fixed for all animals (Gain: 90, Near: -15, Far: 00, Dynamic: 66). On the right side the 10^{th} to 12^{th} intercostal space was clipped then transmission gel was applied and examination was performed from the dorsal to the ventral by using a 3.5 MHz convex probe (15).

Digital analysis

Pictures were saved to be used for quality analysis normal liver, mild, moderate and severe fatty liver were considered according to Komeilian *et al*. (23) and quantity analysis was conducted using Matlab 2020a programming language, in which the image is analyzed, and the required location of the sonar images was determined based on the normal images. Image processing methods were employed to transform the colored images into gray, then white and black and to calculate the percentage of white color that indicates injury. (0=normal, 1-10=mild, 11-20 =moderate, 21-50=severe); the higher the percentage, the higher the injury was. The system was linked to an Excel program that stores the outputs, and this file can be opened whenever a new image is analyzed. Thus, a database was built for all the tested cases.

Enzyme-linked immunosorbent assay

Blood samples (5 ml) of were collected from the milk vein, then serum was separated using gel tube. The level of β-hydroxybutyrate (BHB) was estimated by means of using ELISA kit according to the manufacture procedure (bioassay technology laboratory, china).

Statistical analysis

Data of ELISA were offered as a mean ± standard error and the comparison between diseased and control animals was conducted by using IBM SPSS statistic 21.

Results

Results indicated that out of 50 buffaloes, 15 animals were normal and by the use of ultrasound, they showed normal liver characterized by homogenously equally-distributed weak echoes with clearly visible small and large vessels (Figure 1). On the contrary, the other 35 buffaloes suffered from a decrease in milk production, loss of appetite after parturition. They had a fatty liver and the digital image and qualitative analysis of ultrasound revealed a total of 35 diseased buffaloes. Results indicated that, 20 buffaloes suffered from mild fatty liver and showed a mild increase in parenchyma echogenicity, unclear small vessels, and clear large vessels (Figure 2), while 8 buffaloes had a moderate fatty liver Where it showed an increase in echogenicity with unclear the small and large vessels (Figure 3). Furthermore, 7 buffaloes suffered from the severe fatty liver in which the parenchyma was severely echogenic and the edge is barely visible (Figure 4).

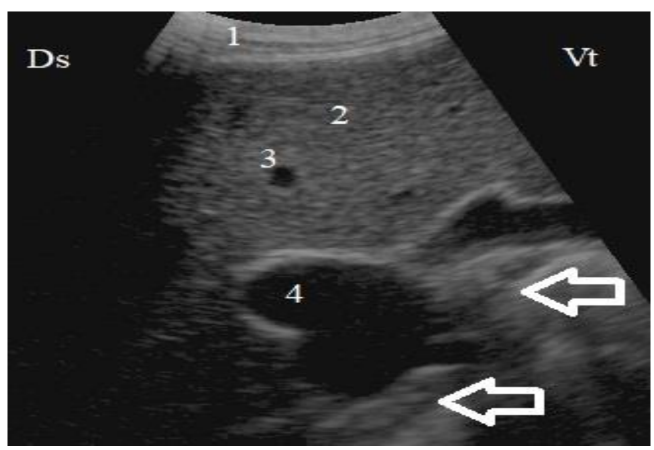

Figure 1: Ultrasonogram of liver from the twelfth intercostal space. The abdominal wall (1), liver (2), small vessel (3), portal vein (4), ventral edge of liver (arrow), Ds: dorsal, Vt: ventral.

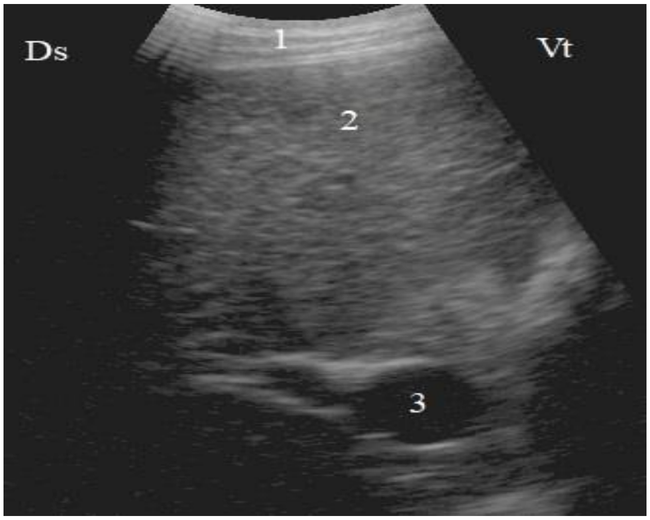

Figure 2: Ultrasonogram of the liver of a buffalo with mild fatty liver. The abdominal wall (1), liver (2), portal vein (3), Ds: dorsal, Vt: ventral.

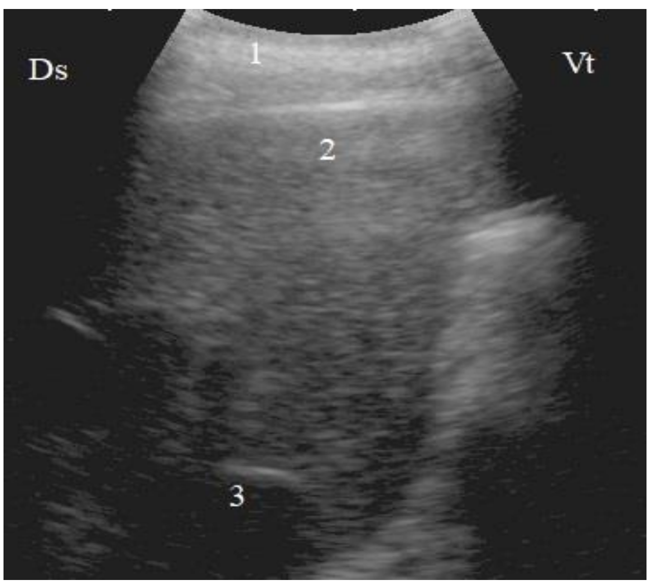

Figure 3: Ultrasonogram of the liver of a buffalo with moderate fatty liver. The abdominal wall (1), liver (2), portal vein (3), Ds: dorsal, Vt: ventral.

Quantitative analysis of total 35 abnormal hepatic images showed 20, 7 and 8 mild, moderate and severe fatty liver respectively, depending on the percentage of white dots in the hepatic image, (Figure 5). From the results of ELISA, there were a significant difference at $P<0.05$ between the mean concentration of BHB of normal buffaloes 0.409 mmol/L and fatty liver diseased buffaloes 0.995 mmol/dL. Furthermore, the mean concentration of BHB in mild, moderate and severe fatty liver were 0.735, 0.923, 1.708 mmol/L respectively with a significant deference between them at $P<0.05$ (Table 1).

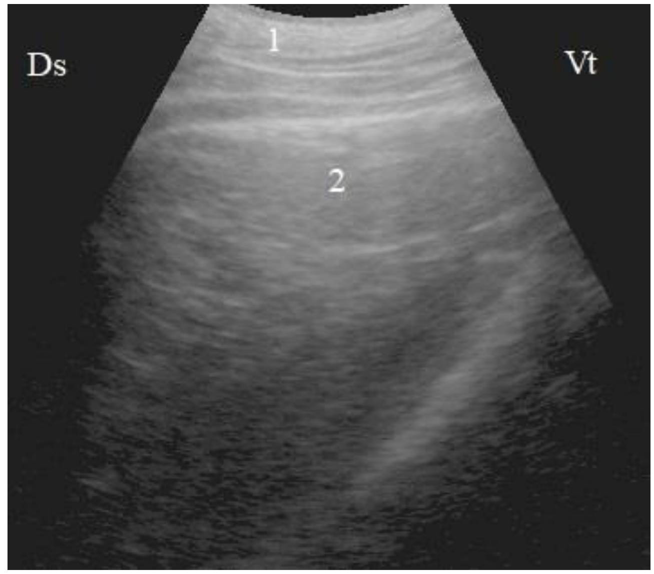

Figure 4: Ultrasonogram of the liver of a buffalo with sever fatty liver. The abdominal wall (1), liver (2), Ds: dorsal, Vt: ventral.

Figure 5: Qualitative image digital analysis of hepatic parenchyma by using fatty-liver software. Red rectangle: area of analysis.

Table 1: Concentration of BHB in normal and diseased buffaloes

Buffaloes	BHB concentration (Mean±SD)		
Normal	0.409±0.01		
Diseased	0.995±0.02 *		
	Mild (N=20)	Moderate (N=7)	Sever (N=8)
	0.735 ± 0.20^{a}	0.923 ± 0.01^{b}	1.708 ± 0.44^{c}

* Significant at $P<0.05$, $^{a-c}$ different letter means significant at $P<0.05$.

Discussion

Results of the current study indicated that Iraqi buffaloes have developed fatty liver and show clinical signs of decrease performance, these results are similar to those recorded by Abdelaal *et al.* (13), fatty liver disease cause severe economic losses due to decrease animals production with morbidity rate may reach 25% and case fatality rate about 90% (3).

There are many methods for diagnosis fatty liver as biochemical analysis, liver biopsy and image analysis (16) the accuracy of biochemical tests may have affected by damage of other tissues or organ lead to valid results, furthermore the liver biopsy should perform with animal under anesthesia and can produce sever liver damage, on the other hand the ultrasonography provide actual hepatic lesions by using real time imaging (24). Results of the qualitative ultrasound image analysis revealed that 20, 8 and 7 buffaloes showed mild, moderate and severe fatty liver respectively, these results agree with (15). The most characteristic changes by ultrasound are the bright pattern, blurring of edges, vascular blurring and deep attenuation (16). The change in hepatic echo patterns due to the presence of fat, which causes difference in hepatic structure, these changes can easily diagnose by ultrasound with a sensitivity of about 90% and a very low false positive (25).

Results of quantitative image analysis show that 35 abnormal images of hepatic parenchyma include 20 mild, 8 moderate and 7 severe fatty liver. These results are in agreement with (6), Buffaloes did not have early pathological changes in their hepatic parenchyma and this made it more accurate to detect mild cases compared to the qualitative analysis and so it should consider a practicable method for detecting fatty liver in buffaloes (13). The estimates of BHB consider as an efficient method for detection of energy balance in cows and buffaloes (2). ELIZA results shows a significant increase in BHB level in diseased buffalo 0.995 mmol/L compared to control group 0.409 mmol/L and these results are consistent with Tharwat (15), NEFA begin increasing from day of parturition due to stress of birth and reach a highest level during the first month post parturition which indicate inability of liver to utilize these metabolites, on the other hand a feeding low quality or quantity diet considered an important predisposing factor (3). From results of ELISA, there were three levels of fatty liver disease in Iraqi female buffaloes and most of them 20 (75.14%) animals within the mild level, this result agrees with Tharwat (15) the difference in these three levels depend on the type of feeding and management practice.

Conclusion

This study indicates that the use of ELIZA can predicts fatty liver stages at laboratory and most of buffaloes have a mild form, which makes clinical diagnosis more difficult practically. Therefore, the use of ultrasound as a noninvasive technique provide an optimal diagnosis of fatty liver and can detect the severity by use Fatty_Liver software.

Acknowledgement

This article was supported by the College of Veterinary Medicine, University of Mosul, Iraq. The authors extend their sincere thanks and gratitude to the Deanship of the College of Veterinary Medicine, University of Mosul for their support in the completion of this study.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

1. Randhawa SNS, Ranjan R, Singh R. Diagnosis and management of negative energy balance and associated production diseases in bovines. Intas Polivet. 2014;15(II):497-503 [available at]
2. Bobe G, Young JW, Beitz DC. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J Dairy Sci. 2004;87(10):3105-3124. DOI: 10.3168/jds.S0022-0302(04)73446-3
3. Constable PD, Blood DC, Radostits OM. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 11th ed. St. Louis: Elsevier; 2017. 1716-1721 p.
4. Al-Mahmood SS, Al-Sabaawy HB. Fasciolosis: Grading the histopathological lesions in naturally infected bovine liver in Mosul city. Iraqi J Vet Sci. 2019;33(2):379-387. DOI: 10.33899/ijvs.2019.125546.1066
5. Al-Mahmood S, Farhan AM, Daoud ZS, Hamed OS. Pathological study of liver lesions in cattle slaughtered at Kirkuk province abattoir. Iraqi J Vet Sci. 2017;28(1):7-16. DOI: 10.33899/ijvs.2017.126713
6. Amniattalab A, Ghalandarzadeh B. Prevalence and pathological evaluation of hepatic fatty change in cattle slaughtered at Urmia Abattoir, Northwest Iran. Iraqi J Vet Sci. 2019;31(1):45-50. DOI: 10.33899/ijvs.2019.125518.1040
7. Singh R, Randhawa SS, Randhawa CS. Hepatic lipidosis in transition buffaloes in relation to back fat thickness, hemato-biochemical and mineral profile. Indian J Anim Res. 2017:30. DOI: 10.18805/ijar.B-3306
8. Rafia S, Taghipour-Bazargani T, Asadi F, Vajhi A, Bokaie S. Evaluation of the correlation between serum biochemical values and liver ultrasonographic indices in periparturient cows with different body condition scores. Am J Vet Res. 2012;73(6):830-7. DOI: 10.2460/ajvr.73.6.830
9. Biller DS, Kantrowitz B, Miyabayashi T. Ultrasonography of diffuse liver disease: A review. J Vet Intern Med. 1992;6:6. DOI: 10.1111/j.1939-1676.1992.tb03154.x
10. Aliraqi OM. Ultrasonography of left displaced abomasum in local cattle breed. Iraqi J Vet Sci. 2021;35(1):139-44. DOI: 10.33899/ijvs.2020.126447.1328
11. Abdelaal AM, Gouda SM, Tharwat M. Clinico-biochemical, ultrasonographic and pathological findings of hepatic abscess in feedlot cattle and buffaloes. Vet World. 2014;7(5):306-10. DOI: 10.14202/vetworld.2014.306-310
12. Tharwat M. Ultrasonography of the liver in healthy and diseased camels (*Camelus dromedaries*). J Vet Med Sci. 2020;82(4):399-407. DOI: 10.1292/jvms.19-0690
13. Abdelaal AM, Abd El Raouf M, Aref MA, Moselhy AA. Clinical and ultrasonographic investigations of 30 water buffaloes (*Bubalus bubalis*) with hepatomegaly. Vet World. 2019;12(6):789-95. DOI: 10.14202/vetworld.2019.789-795

14. Hussein HA, El-sheikh AK, Degheidy NS, Tharwat M. Diagnostic investigations for fatty liver syndrome in Egyptian buffaloes. 2014;6. DOI: 10.5829/idosi.gv.2014.12.05.8387
15. Tharwat M. Ultrasonography as a diagnostic and prognostic approach in cattle and buffaloes with fatty infiltration of the liver. Pol J Vet Sci. 2012;15(1):83-93. DOI 10.2478/v10181-011-0118-4
16. Acorda JA, Yamada H, Ghamsari SM. Comparative evaluation of fatty infiltration of the liver in dairy cattle by using blood and serum analysis, ultrasonography, and digital analysis. Vet Q. 1995;17(1):12-4. DOI: 10.1080/01652176.1995.9694522
17. Thijssen JM, Weijers G, de Korte CL. Objective performance testing and quality assurance of medical ultrasound equipment. Ultrasound Med Biol. 2007;33(3):460-71. DOI: 10.1016/j.ultrasmedbio.2006.09.006
18. Thijssen JM. Ultrasonic speckle formation, analysis and processing applied to tissue characterization. Pattern Recognit Lett. 2003;24(5):659-75.DOI: 10.1016/S0167-8655(02)00173-3
19. Reeder SB, Sirlin CB. Quantification of liver fat with magnetic resonance imaging. Magn Reson Imaging Clin N Am. 2010;18(3):337-57. DOI: 10.1016/j.mric.2010.08.013
20. Dahl MO, Hamdoon OK, Abdulmonem ON. Epidemiological analysis for medical records of veterinary teaching hospital, university of Mosul during 2017 to 2019. Iraqi J Vet Sci. 2021;35(3):541-548. DOI: 10.33899/ijvs.2020.127141.1468
21. Rahawy MA. Study on the post-partum disorders and their relationship with the reproductive performance in Iraqi cow-buffaloes. Iraqi J Vet Sci. 2021;35(2):313-317. DOI: 10.33899/ijvs.2020.126771.1387
22. Al-Watar, BD, Lazim, E, Al-Hyani OH. Creatine kinase and C reactive protein as an indicator for tissue damage in the retained placenta in cows. Iraqi J Vet Sci. 2021;35(1):163-167. DOI: 10.33899/ijvs.2020.126496.1338
23. Komeilian MM, Sakha M, Nadalian MG, Veshkini A. Hepatic ultrasonography of dairy cattle in postpartum period: finding the sonographic features of fatty liver syndrome. Aust J Basic Appl Sci. 2005;5(8):701-706. [available at]
24. Foster KJ, Dewbury KC, Griffith AH, Wright R. The accuracy of ultrasound in the detection of fatty infiltration of the liver. Br J Radiol. 1980;53(629):440-2. DOI: 10.1259/0007-1285-53-629-440
25. Acorda JA, Yamada H, Mehdi Ghamsari S. ultrasonographic features of diffuse hepatocellular disorders in dairy cattle. Vet Radiol Htmlent Glyphamp Asciiamp Ultrasound. 1994;35(3):196-200.DOI: 10.1111/j.1740-8261.1994.tb01592.x

تحليل صور الموجات فوق الصوتية للكبد الدهني في الجاموس في الموصل، العراق

زينة حازم حسن[١]، أسامة موفق العراقي[١]
و إيلاف أسامة عبد المجيد[٢]

[١]فرع الطب الباطني، كلية الطب البيطري، [٢]قسم علوم الحاسبات، كلية علوم الحاسبات والرياضيات، جامعة الموصل، الموصل، العراق

الخلاصة

هدفت الدراسة إلى معرفة إمكانية استخدام التحليل الرقمي النوعي والكمي لصور الكبد بالموجات فوق الصوتية للكشف عن الكبد الدهني وتصنيف شدته ولتقدير تركيز محتوى بيتاهيدروكسي بيوتريت في الدم في الحيوانات السليمة والمريضة. تمت دراسة ٥٠ جاموسة (١٥ طبيعية سريريا و ٣٥ تعاني من انخفاض إنتاج الحليب وفقدان الشهية وفقدان وزن الجسم) بعد ٢-٤ أسابيع من الولادة. تم فحص الجاموس بواسطة الموجات فوق الصوتية باستخدام مسبار محدب ٣,٥ ميجا هرتز. تم حفظ صور الموجات فوق الصوتية للتحليل النوعي والكمي، باستخدام برنامج الكبد الدهني (يعتمد لغة البرمجة مات لاب). بناء على التحليل الرقمي لصور الموجات فوق الصوتية، تم تصنيف الجاموس المصاب بالكبد الدهني إلى ٢٠ إصابة خفيفة و٧متوسطة و ٨ شديدة. أشارت النتائج إلى أن تركيز بيتاهيدروكسي بيوتريت بواسطة اختبار المقايسة المناعي المرتبط بالإنزيم كان ٠,٤٠٩ و ٠,٧٣٥ و ٠,٩٢٣ و ١,٧٠٨ مليمول/ديسيلتر في الجاموس الطبيعي، والجاموس المصاب إصابة خفيف، متوسطة وشديدة على التوالي ومع وجود فرق معنوي بين الحيوانات السليمة والمريضة. وقد تم التوصل إلى أن الجاموس في الموصل يعاني من الكبد الدهني وان التحليل الرقمي للصور بالموجات فوق الصوتية كانت طريقة مناسبة للتشخيص السريري، علاوة على ذلك كان تقدير بيتاهيدروكسي بيوتريت طريقة مناسبة للتشخيص المختبري.