Early assessment of nonalcoholic fatty liver disease using multiparametric ultrasound imaging

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approach and evaluate its use for assessing NALFD, thereby introducing a surrogate biomarker comparable to liver biopsy. The novel method combines contrast-enhanced ultrasound (CEUS), shear wave elastography (SWE), and H-scan ultrasound (US) imaging. This approach integrates information related to liver tissue perfusion, viscoelasticity, and US scatterer size. Using Sprague-Dawley rats that were fed either control or a methionine and choline deficient (MCD) diet (***N* = 6 per group), mpUS imaging was performed at week 0 (baseline), 2 and 6. Thereafter, animals were euthanized and livers excised for histological processing and analysis. *In vivo* mpUS results from control and diet fed animals revealed that all parametric measures were statistically different at 6 weeks. Histological results revealed the presence of steatosis and mild fibrosis in animals fed the MCD diet. Overall, mpUS imaging was shown to be a promising approach for the early assessment of NAFLD.**

Keywords—contrast-enhanced ultrasound, fatty liver disease, H-scan ultrasound, shear wave elastography, tissue characterization.

I. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease among adults in the United States and affects between 80 to 100 million [1]. NAFLD comprises a wide spectrum of liver pathology ranging from non-alcoholic steatohepatitis (NASH), that can develop into liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [2]. Liver biopsy is commonly used as the reference standard method to grade liver cell injury from NASH [3]. However, liver biopsy is invasive, associated with sampling errors, interrater and intrarater variability

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PDFF). MRE provides a quantitative measure of liver stiffness, which is based on an analysis of shear waves induced in the liver by low-frequency vibrations applied to the abdominal wall. Measurements have been shown to correlate with fibrosis stage [5]. MRI-PDFF is a quantitative imaging biomarker of steatosis that can accurately estimate liver fat content [6]. Despite strengths, these techniques may not be practical or cost-effective for clinical screening for early stage NAFLD. On the other hand, ultrasound (US) has evolved as a promising and cost-effective imaging method for early steatosis grading [7], [8].

II. MATERIALS AND METHODS

A. Animal Preparation

Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Dallas. Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were randomly divided into two groups: control and diet (N = 6 per group). Control animals were fed standard chow, whereas diet animals were fed a special methionine and choline deficient (MCD) chow known to induce NAFLD. Before imaging, the abdomen and surrounding area were shaved with electric clippers and depilated (Nair, Church & Dwight Co, Ewing, NJ). During all procedures, animals were controlled using isoflurane anesthesia and placed on a heating pad to help maintain core body temperature.

B. mpUS Imaging

In vivo mpUS imaging of the liver in all animals occurred at baseline and again after 2 and 6 weeks of feeding with the control or MCD diet. SWE was performed using a Vantage 256 US system (Verasonics Inc, Kirkland, WA) equipped with an L11-4v linear array transducer. The SWE pulse sequence consisted of 3 rapid push beams applied using a pulse frequency of 5.2 MHz, aperture size of 64 elements, pulse length of 230 µs, and axial spacing between push beams of 2 mm. After inducing shear wave propagation in the liver parenchyma, ultrafast plane wave imaging was used to track the shear wave propagation (frame rate of 10 kHz). A 2D algorithm was used to estimate tissue displacements from the beamformed in-phase quadrature (IQ) data [20]. Thereafter, a 2D Fourier transform was applied on the tissue displacements to estimate SWS and SWA.

H-scan US imaging was performed using a Vevo 3100 system (FUJIFILM VisualSonics Inc, Toronto, Canada) equipped with an MX201 linear array transducer (center frequency of 15 MHz). Beamformed radiofrequency (RF) data was collected in the liver parenchyma and processed using a pair of convolution filters constructed using Gaussian-weighted Hermite polynomial functions of order 2 and 8 (denoted ψ_2 and ψ_8, respectively) [21]. The relative strength of these filter outputs was normalized by the signal energy $\sqrt{\psi_2^2 + \psi_8^2}$ and then the lower frequency backscattered US signals (ψ_2) were assigned a red (R) channel and higher frequency components (ψ_8) to a blue (B) channel. The envelope of the original unfiltered data was assigned to a green (G) channel to complete the RGB color map and H-scan image display. H-scan US image intensity was calculated as a ratio of the B channel to the sum of the R and B channel components and describes relative scatterer size (rSS).

CEUS was also performed using the Vevo 3100 US system but in a MB-sensitive nonlinear imaging mode (center frequency of 12.5 MHz). After administering a slow bolus injection of MB contrast agent via a place tail vein catheter (50 µL; Definity, Lantheus Medical Imaging, N Billerica, MA), a temporal sequence of CEUS image were acquired (about 1 min). After placement of a region-of-interest (ROI) in the liver parenchyma or circumscribing the inferior vena cava (IVC), a mean time-intensity curve was constructed. From each, the peak enhancement (PE) and wash-in rate (WIR) parameters were extracted and represent surrogate measurements of blood volume and flow rate, respectively. CEUS parameters from the liver parenchyma were normalized by those from the IVC. Note that CEUS image was performed last in the mpUS sequence as the presence of any residual MBs in circulation could induce bioeffects if exposed to high-intensity US during SWE imaging.

MRE and MRI-PDFF assessments is the high cost associated with MRI scanners along with the technical expertise required to perform and interpret readings.

US is an alternative imaging method for early screening of NAFLD. Steatosis can be classified using B-mode US images and the assessment of liver echogenicity that is linked to fat accumulation. However, B-mode US has relatively poor interobserver agreement due to its subjective nature, along with reduced sensitivity in detecting mild hepatic steatosis. In recent years, several different US biomarkers have been developed assess a range of soft tissue properties. In this study, we introduced a new mpUS method that uses SWE parameters to access liver viscoelasticity, CEUS to capture features of liver vascularity, and H-scan US to obtain tissue microstructure information.

SWE is a noninvasive imaging technique that uses both US and low-frequency elastic waves to quantify biomechanical

significantly different by 6 weeks. Further, these US imaging results were qualitatively confirmed by histological findings that revealed the MCD fed animals had considerable levels of steatosis. Future work will examine the mpUS data classification accuracy and a more detailed comparison to histological