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● *Original Contribution*

EVALUATION OF NON-ALCOHOLIC FATTY LIVER DISEASE USING ACOUSTIC RADIATION FORCE IMPULSE IMAGING ELASTOGRAPHY IN RAT MODELS

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Abstract—The aim of this study is to evaluate the utility of acoustic radiation force impulse (ARFI) elastography for assessing hepatic fibrosis stage and non-alcoholic fatty liver disease (NAFLD) severity, as well as the relationship among hepatic histologic changes using shear wave velocity (SWV). Animal models with various degrees of NAFLD were established in 110 rats. The right liver lobe was processed and embedded in a fabricated gelatin solution (porcine skin). Liver mechanics were measured using SWV induced by acoustic radiation force. Among the histologic findings, liver elasticity could be used to differentiate normal rats from rats with simple steatosis (SS) as well as distinguish SS from non-alcoholic steatohepatitis (NASH), with areas under the receiver operating characteristic curves (AUROC) of 0.963 (95% confidence interval = 0.871–0.973) and 0.882 (95% confidence interval = 0.807–0.956), respectively. For NAFLD rats, the diagnostic performance of ARFI elastography in predicting significant fibrosis (F ≥ 2) had an AUROC of 0.963. For evaluating steatosis severity, we found a progressive increase in ARFI velocity proportional to steatotic severity in NAFLD rat models, but we observed no significant differences for steatotic severity after excluding the rats with fibrosis. ARFI elastography may be used to differentiate among degrees of severity of NAFLD and hepatic fibrotic stages in NAFLD rat models. (E-mail: chenxin@szu.edu.cn)

Key Words: **Acoustic radiation force impulse elastography, Fibrosis, Non-alcoholic fatty liver disease, Shear wave velocity, Steatosis, Non-alcoholic steatohepatitis.**

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and is a major global health problem (Starley et al. 2010). NAFLD is defined as the accumulation of lipid deposits in hepatocytes not resulting from excessive alcohol use (Liou and Kowdley 2006). NAFLD represents a spectrum of diseases ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH), which can progress to liver fibrosis, cirrhosis and hepatocellular carcinoma (Angulo 2002; Harrison et al. 2003). Therefore, it is clinically important to distinguish SS from NASH and to assess the severity of hepatic fibrosis for risk-stratified management of patients with NAFLD.

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Liver biopsy remains the gold standard for diagnosing NAFLD, but it is invasive and associated with severe complications in 0.3%–3.0% of cases and leads to death in 0.01% of cases (Pagadala and McCullough 2012). Furthermore, it depends on surgical expertise and is subject to sampling error because of the small size of biopsy samples (Grandison and Angulo 2012; Pagadala and McCullough 2012). Therefore, a simple and non-invasive method for confirming NAFLD is of major clinical interest.

Various imaging methods have been proposed as non-invasive alternatives to liver biopsy, including ultrasonography (US), transient elastography (TE), acoustic radiation force impulse (ARFI) elastography and magnetic resonance imaging (MRI) (Cosgrove et al. 2013; Ferraioli et al. 2015; Jeong et al. 2014; Machado and Cortez-Pinto 2013; Piscaglia et al. 2013). Recently these imaging methods have been evaluated for their usefulness for diagnosing NAFLD (Cassinotto et al.

2016; Deffieux et al. 2015; Fierbinteanu Braticevici et al. 2013; Ghoshal et al. 2012; Guzmán-Aroca et al. 2012; Kang et al. 2015; McPherson et al. 2009; Petta et al. 2015; Sasso et al. 2016; Yoneda et al. 2010).

ARFI elastography is a technique that measures shear wave velocity (SWV), which can provide information on the mechanical properties of soft tissue, using high-intensity acoustic pulses to generate localized displacements in tissue (Sarvazyan et al. 1998; Zhai et al. 2008). It has the advantage of being integrated into a conventional US system; therefore, it is useful for real-time evaluation of stiffness during standard US examinations of the liver (Lupsor et al. 2009). Although previous ARFI studies indicated that assessing liver fibrosis was feasible for patients with viral hepatitis (Ebinuma et al. 2011; Friedrich-Rust et al. 2009; Goertz et al. 2010), the efficacy of SWV for evaluating NAFLD has not been determined. NAFLD accompanies various histologic abnormalities, including hepatic steatosis, inflammation and fibrosis, which may potentially affect shear wave propagation (Deffieux et al. 2015; Jeong et al. 2014; Kang et al. 2015; Machado and Cortez-Pinto 2013; Piscaglia et al. 2013). Previous NAFLD studies suggested that SWV values were significantly correlated with fibrotic liver stages for patients with NAFLD, but results about the relationship among SWV and steatosis and inflammation severity grades are still conflicting (Cassinotto et al. 2013, 2016; Deffieux et al. 2015; Ebinuma et al. 2011; Fierbinteanu Braticevici et al. 2013; Guzmán-Aroca et al. 2010, 2012; Kang et al. 2015; Palmeri et al. 2011; Petta et al. 2015; Wong et al. 2010; Yoneda et al. 2010). Specifically, for evaluating steatotic severity grade, previous studies using the ARFI technique omit evaluations of the influence of fibrosis on steatosis (Cassinotto et al. 2013, 2016; Fierbinteanu Braticevici et al. 2013; Guzmán-Aroca et al. 2012; Nightingale et al. 2015; Petta et al. 2015; Yoneda et al. 2010).

Our previous study focused on a rat model of steatosis (Guo et al. 2017) and this study expanded those findings to an NAFLD model. A more complex histologic scoring system was used to evaluate NAFLD for the purpose of using ARFI for differentiating NAFLD histologic subtypes and for diagnosing fibrotic stages with NAFLD. In addition, we also studied the relationship of various histologic changes with liver SWV in an NAFLD model.

MATERIALS AND METHODS

Animal model

Liver NAFLD was induced in male Sprague-Dawley (SD) rats (Guangdong Medical Laboratory Animal Center, Guangdong, China), weighing 170 ∼ 220 g. Rats were housed in sterile isolated cages with a 10–14 h light and dark cycle at a constant temperature (20–26°C) and humidity (40%–70%). To obtain rats with varying degrees of NAFLD severity, 110 SD rats were randomized into 4 groups. The first group (n = 18) was provided a standard diet with sterilized food and water. The second group (n = 57) was on a special high-fat emulsion diet (20% lard, 10% cholesterol, 2% sodium cholate, 0.5% propylthiouracil and 30% fructose), once a day at 1 mL/100 g per rat weight, for various time periods (2, 4, 6 and 8 wk) to induce various grades of steatosis. The third group (n = 22) was provided with the same high-fat emulsion diet for 8 wk. Furthermore, 50% carbon tetrachloride (CCl_4) in olive oil was injected subcutaneously 2 × wk at 0.6 mL/100 g per rat weight during the first two weeks and 0.3 mL/100 g per rat weight in the remaining wk. The fourth group (n = 13) was provided a standard diet and injected with CCl_4 for 4 wk to induce liver fibrosis. After the gavage was completed, the rats were euthanized, the right lateral lobe of the liver was harvested for ARFI measurements and the other lobes were used for histologic assessment. NAFLD severity was histologically confirmed. All procedures in these studies were approved by the Animal Care Committee of Shenzhen University and the Guangdong Medical Laboratory Animal Center.

Biochemical evaluation

Blood samples (1.5 mL) were extracted from the rats' orbital venous sinus after a 12-h fasting period. Plasma was separated and analyzed for total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using an automatic biochemical analyzer (Type 7020, Hitachi High-Technologies Corporation, Tokyo, Japan).

Histologic assessment

Excised liver tissues were fixed in 10% formalin solution for at least 24 h. After washing and dehydrating, samples were embedded in paraffin and sliced to a thickness of 7 um. Histopathology technicians stained the paraffin slices with Oil Red O (ORO), hematoxylin-eosin (HE) and Masson's trichrome (MT). The slices were analyzed using a microscope (BX41, Olympus, Pittsburgh, PA, USA) by an expert pathologist (experience of 20 y) who was blinded to the study design, treatment groups and data from blood and ultrasound measurements. Histologic analysis was performed according to the scoring system developed by Kleiner's group (Kleiner et al. 2005). The scoring system used was the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of 3 subscores: steatosis (0–3), inflammation (0–3) and hepatocyte ballooning (0–2). On the basis of the NAS score, the final pathologic