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● *Original Contribution*

COULD ULTRASOUND ELASTOGRAPHY REFLECT LIVER FUNCTION?

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Abstract—The purpose of this study was to investigate whether ultrasound elastography reflects liver function reserve relative to liver fibrosis histology. Sixty-five New Zealand rabbits were divided into an experimental group (n = 45) and a control group (n = 20). In the experimental group, liver fibrosis (F1–F4) was induced by subcutaneous injection of carbon tetrachloride. Point shear wave elastography and the indocyanine green (ICG) elimination test were performed for the two groups at 4-wk intervals for 56 wk. The liver stiffness value (LSV) and the ICG retention rate at 15 min (ICGR15) were obtained, and the correlation between them was investigated. The median LSVs of stages F0–F4 were 3.92 kPa (1.91–8.53 kPa), 5.02 kPa (2.39–8.91 kPa), 7.87 kPa (5.21–12.26 kPa), 12.83 kPa (5.92–16.79 kPa) and 16.64 kPa (9.76–29.50 kPa), respectively. The median ICGR15 values of stages F0–F4 were 8.7% (4.8%–15.6%), 10.8% (5.6%–20.3%), 19.2% (12.3%–26.7%), 31.0% (20.9%–41.0%) and 45.6% (22.1%–60.9%). There were significant differences in LSVs and ICGR15 values among the different stages of liver fibrosis ($p < 0.01$). A positive correlation was observed between LSV and ICGR15 ($r = 0.7497$, $p < 0.0001$). A strong correlation was observed between liver stiffness and liver function reserve, indicating ultrasound elastography may reflect liver function reserve in different degrees of liver fibrosis. (E-mail: luoyanddoc@163.com) **© 2018 Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Point shear wave elastography, Liver stiffness, Indocyanine green elimination test, Liver function reserve, Liver fibrosis.**

INTRODUCTION

Liver fibrosis is the pathologic process of abnormal proliferation of fibrous tissue in the liver in most chronic liver diseases, and is accompanied by a variety of changes in function and stiffness (Fattovich et al. 2008; Younossi et al. 2011). In the development of fibrosis, impaired liver function reserve is a marked pathophysiologic change. Evaluation of liver function reserve before liver resection or transplantation is clinically significant (Clavien et al. 2007). Liver function reserve is also important in determining the prognosis in patients with cirrhosis (Schwartz et al. 2010). An established approach for assessing liver function reserve is the indocyanine green (ICG) elimination test. ICG is a cyanine dye taken up by hepatocytes and eliminated from blood flow exclusively by the liver (Clavien et al. 2007). The ICG elimination test has been reported to be quite sensitive for detecting early hepatocyte abnormalities (Stintzing et al. 2009). In comparison, conventional liver function tests such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) suffer from low sensitivity and specificity, especially in the detection of compensated liver insufficiency. However, ICG measurement has significant buy-in costs related to equipment purchases. In addition to this charge, the cost per measurement (corresponding to the cost of fluorescent dye) is about 45€ (Levesque et al. 2016). Moreover, although ICG is generally very well tolerated, its use is not advisable in patients with an iodine allergy or thyrotoxicosis because of its iodine component. In extremely rare cases, an ICG injection can cause nausea and an anaphylactic reaction (incidence of approximately 1:40,000) (Hunton et al. 1960).

Gray-scale and color Doppler ultrasound are routine non-invasive ultrasound techniques widely used for detecting liver cirrhosis, but are not sufficiently sensitive for diagnosing early fibrosis. Ultrasound elastography, based on the measurement of the velocity of an elastic shear wave

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propagating through the liver, provided a possibility for the detection of early liver fibrosis (Bamber et al. 2013). A variety of ultrasound elastography techniques, such as transient elastography (TE), point shear wave elastography (pSWE) and real-time shear wave elastography (2-D-SWE) imaging, have been investigated or applied for quantitative assessment of liver fibrosis (Cosgrove et al. 2013). pSWE is less influenced by ascites and obesity compared with TE and is performed under the guidance of conventional ultrasound (Bamber et al. 2013). pSWE has been reported to be clinically beneficial in assessing liver fibrosis with good reproducibility (Ling et al. 2013; Lu et al. 2016).

Although the ICG elimination test or pSWE has been investigated or applied in the evaluation of liver fibrosis, the correlation of liver stiffness with liver function reserve has not been fully investigated. Therefore, this study adopted an experimental rabbit model of liver fibrosis to observe the stiffness changes of liver at different stages of fibrosis by pSWE and to evaluate whether liver stiffness reflects changes in liver function reserve.

METHODS

Animal model

The use of experimental animals was approved by the animal ethics committee of the West China Hospital, Sichuan University. All experiments complied with the protocols and guidelines for the humane treatment of animals for research and teaching. A total of 80 New Zealand rabbits (mean age ± standard deviation [SD] = 120.5 ± 20.5 d), each of which weighed about 2.5 kg at the beginning of this study, were enrolled in the study. The rabbits were randomly divided into a control group (n = 20) and an experimental group (n = 60). For the animals in the experimental group, liver fibrosis was induced by subcutaneous injection of 0.3 mL/kg wt 50% carbon tetrachloride (CCl_4) in olive oil as a vehicle, once per wk. CCl_4 was purchased from Beijing Chemical Reagent Company (Beijing, China). For the control group, the animals received 0.3 mL/kg olive oil by subcutaneous injection once per wk. All rabbits were numbered (1, 2, 3, …, 80). Every 4 wk, 3–6 rabbits from the experimental group and 1 or 2 rabbits from the control group were randomly selected for ultrasound, conventional liver function tests and the ICG elimination test. Rabbits that died before or during these procedures were excluded, and the rest were sacrificed immediately after these procedures to obtain liver specimens.

pSWE examination of the liver

All pSWE examinations were performed with an iU22 ultrasound system (Royal Philips, Netherlands) equipped with C5-1 (curved 1–-5 MHz) transducer. The rabbits were anesthetized by intramuscular administration of 0.2 mL/kg zolazepam/tiletamine (Zoletil, Virbac Korea, Seoul, Korea) and 1–2 mg/kg xylazine hydrochloride (Rompun, Bayer Korea, Seoul, Korea). After anesthesia, the rabbit was placed in the supine position, and its upper abdomen was prepared shaved for liver ultrasound scanning. The liver was initially examined with gray-scale ultrasound using a C5-1 probe. The liver lobe was displayed with subcostal (no additional pressure of the transducer is applied) scanning. With the transducer maintained in the same place and the liver in the selected section steadily displayed, the sonographic unit was then switched to Elast Point Quantification (ElastPQ) mode. The size of the region of interest (ROI) box for Elast PQ was depth dependent with 0.5 × 1.5 cm at a depth of 4 cm. The penetration depth for all measurements ranged from 2 to 7 cm. The ROI was randomly placed on the liver parenchyma for stiffness measurement, taking care to avoid the large vessels and capsule (Fig. 1). An IQR/M (ratio of interquartile range to median) < 30% is considered a successful measurement. For each rabbit, 10 measurements were carried out. The average successful measurement rate was about 70%. The liver stiffness value (LSV) for each rabbit was obtained. All pSWE examinations were carried out by an experienced (>5 y) sonographer who was blinded to the animal information and pathologic results.

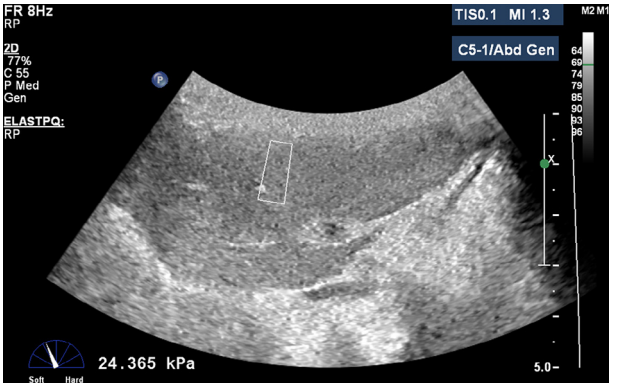

Fig. 1. *In vivo* stiffness measurements. Stiffness measurement of one liver lobe displayed over B-mode ultrasound images. The *white box* represents the region of interest for stiffness measurement.

ICG elimination test and conventional liver function test

After ultrasound examination, ICG and conventional liver function tests were performed. ICG was dissolved in saline water to a final concentration of 1.25 mg/mL. ICG was injected at a dose of 0.1 mL/kg *via* the auricular vein. Serum was collected before and 15 min after the ICG injection to determine the ICG retention rate at 15 min (ICGR15). Each serum sample was diluted to one-fourth of the concentration with saline, and the concentration of ICG in the specimens was analyzed by spectrophotom-