Ultrasound in Med. & Biol., Vol. 40, No. 4, pp. 739–746, 2014

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2013.08.011

● *Original Contribution*

EFFECTS OF VASCULARITY AND DIFFERENTIATION OF HEPATOCELLULAR CARCINOMA ON TUMOR AND LIVER STIFFNESS: *IN VIVO* AND *IN VITRO* STUDIES

Wenwu Ling,* Qiang Lu,* Changli Lu,† Jierong Quan,* Lin Ma,* Jiawu Li,* Du He,† Jianping Liu,† Jiaying Yang,‡ Tianfu Wen,‡ Hong Wu,‡ Hongguang Zhu,§ and Yan Luo*

*Department of Ultrasound, West China Hospital of Sichuan University, Chengdu, China; †Department of Pathology, West China Hospital of Sichuan University, Chengdu, China; ‡Department of Hepatobiliary Surgery, West China Hospital of Sichuan University, Chengdu, China; and §Department of Pathology, Shanghai Medical College, Fudan University, Shanghai, China

(*Received* 22 *October* 2012; *revised* 12 *August* 2013; *in final form* 13 *August* 2013)

Abstract—Tissue stiffness has been found to be a useful predictor of malignancy in various cancers. However, data on the stiffness of hepatocellular carcinomas (HCCs) and their background livers are contradictory. The aim of this study was to investigate the effects of vascularity and histologic differentiation on HCC stiffness. Elastography point quantification (ElastPQ), a new shear wave-based elastography method, was used to measure liver stiffness *in vivo* in 99 patients with pathology-proven HCC. Lesion vascularity was assessed using contrast-enhanced ultrasound, computed tomography and/or magnetic resonance imaging. The association of HCC vascularity and differentiation with liver stiffness was determined. In addition, *in vitro* stiffness of 20 of the 99 surgical HCC specimens was mechanically measured and compared with *in vivo* measurements. We found that *in vivo* stiffness was significantly higher than *in vitro* stiffness in both HCCs and their background livers ($p < 0.0001$). Moreover, significantly higher stiffness was observed in hyper-vascular and poorly differentiated lesions than in hypo-vascular ($p = 0.0352$) and moderately to well-differentiated lesions ($p = 0.0139$). These *in vivo* and *in vitro* studies reveal that shear wave-based ultrasound elasticity quantification can effectively measure *in vivo* liver stiffness. (E-mail: huaxiluoyan@gmail.com) **© 2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound elastography, Elastography point quantification, Liver stiffness measurement, Tissue elastometer, Hepatocellular carcinoma, Tumor vascularity.**

INTRODUCTION

Elasticity or stiffness is the biologic and mechanical property of a soft tissue that depends on its molecular components and internal structure (Humphrey 2003). Organ stiffness changes through pathologic processes (Gao et al. 1996; Wells and Liang 2011). Thus, the standard medical practice of soft tissue palpation is based on qualitative assessment of tissue stiffness (Mahoney and Csima 1982; Ophir et al. 1999; Morikubo 2005). In many cases, despite differences in stiffness, the small size of a lesion and/or its location deep in the body makes its detection by palpation difficult, even impossible.

Recently, a number of ultrasound-based techniques, such as strain imaging (Wells and Liang 2011) and shear wave-based elastography techniques (Heide et al. 2010; Bercoff et al. 2004; Chen et al. 2009), have been developed to estimate the elastic modulus of tissues non-invasively. In strain imaging, external compression is needed to induce tissue deformation, and clinical applications are limited mainly to superficial organs, whereas shear wave-based elastography techniques, such as acoustic radiation force impulse (ARFI) imaging (Heide et al. 2010), shear wave elastography (SWE) (Bercoff et al. 2004) and shear wave dispersion ultrasonic vibrometry (Chen et al. 2009), can quantitatively assess stiffness in deeply situated organs. Very recently, a new shear wave-based elastography technique known as elastography point quantification (ElastPQ) was developed and incorporated into a complete ultrasound system (Xie et al. 2010; Chen et al. 2013).

Tissue stiffness has proved to be a useful predictor of malignancy in breast, prostate and thyroid cancers (Ding et al. 2011; Itoh et al. 2006; Kamoi et al. 2008). Recent studies on liver stiffness indicate sono-elastography

Address correspondence to: Yan Luo, Department of Ultrasound, West China Hospital of Sichuan University, Chengdu 610041, People's Republic of China. E-mail: huaxiluoyan@gmail.com

may be useful in screening for hepatocellular carcinoma (HCC) (Gheorghe et al. 2009; Cho et al. 2010). However, the data on the stiffness of HCCs and their background livers are contradictory (Fahey et al. 2008; Gallotti et al. 2012; Heide et al. 2010). Validating the ability of elasticity quantification to reveal *in vivo* liver stiffness is an important prerequisite for its widespread application.

Our study investigated *in vivo* liver stiffness as assessed with ElastPQ technology in 99 patients with pathology-proven HCC. The association of vascularity and histologic differentiation with HCC stiffness was then determined for the 99 patients. In addition, a tissue elastometer (Egorov et al. 2008) was employed to mechanically measure *in vitro* stiffness in 20 of the 99 surgical liver specimens, and the values obtained were compared with *in vivo* measurements. These *in vivo* and *in vitro* studies allowed us to evaluate the ability of shear wave-based ultrasound elasticity quantification to reveal *in vivo* liver stiffness.

METHODS

Patients and study design

Between March 2011 and May 2012, 99 patients with hepatitis B virus (HBV)-related HCC underwent ElastPQ examination and liver resection surgery in West China Hospital of Sichuan University. All of the HCCs were independently diagnosed by two experienced pathologists (L.L. and D.H.) and were reviewed by an expert gastroenterology pathologist (Z.H.). Moreover, the Scheuer classification (Desmet et al. 1994) was used to stage chronic hepatitis in background liver >2 cm from the lesion. None of the patients had undergone pre-operative chemotherapy, radiotherapy, transarterial chemoembolization or radiofrequency ablation. Characteristics of the patients are summarized in Table 1. Grade I and I/II tumors were characterized as well-differentiated lesions, grade II tumors as moderately differentiated lesions and tumors grade II/III and III tumors as poorly differentiated lesions. For patients with multiple lesions, the largest tumor was chosen as the study subject for data analysis. This study was approved by the Ethics Committee of West China Hospital in Sichuan University, and written informed consent was obtained from all participants.

The study was divided into three different phases: (i) Liver stiffness of 99 patients with HCC was measured before liver resection. (ii) The association of vascularity and histologic differentiation with HCC stiffness was investigated in the 99 patients. (iii) *In vitro* stiffness measurements of 20 of the 99 surgical HCC specimens was compared with their *in vivo* stiffness measurements.

Table 1. Clinical characteristics of study patients

Hepatocellular carcinoma	
Age (mean ± SD)	47 ± 11
Sex	
Male	85 (86)*
Female	14 (14)
Contrast-enhanced imaging†	
Hypo-vascular lesion	27 (27)
Hyper-vascular lesion	72 (73)
Tumor size	
≤3 cm	23 (23)
>3 cm	76 (77)
Multiple tumors	
Yes	17 (17)
No	82 (83)
Differentiation	
Well (I and I/II)	7 (7)
Moderate (II)	54 (55)
Poor (II/III and III)	38 (38)
BCLC stage	
0	2 (2)
A	19 (19)
B	54 (55)
C	24 (24)
Vascular invasion	
Yes	36 (36)
No	63 (64)
Background liver‡	
Normal liver (S0)	3 (3)
Fibrotic liver (S1–S3)	49 (49)
Cirrhotic liver (S4)	47 (47)

BCLC = Barcelona Clinic Liver Cancer (Llovet et al. 2008).
* Number (%).
† Hepatocellular carcinoma was classified into hyper-vascular and hypo-vascular lesions based on its enhanced pattern during the hepatic arterial phase on contrast-enhanced ultrasound (Jang et al. 2007), computed tomography (Nakaura et al. 2008) and/or magnetic resonance imaging (Murakami et al. 2012).
‡ The Scheuer classification (Desmet et al. 1994) was used to stage chronic hepatitis in the background liver, >2 cm away from the lesion.

B-mode ultrasound examination

Before ElastPQ examination, all participants underwent B-mode liver ultrasound scanning. Examinations and diagnoses were conducted by two ultrasound physicians (Y.L. and Q.L.), each with more than 5 y of experience in hepatic applications. The ultrasound scan was performed with an iU22 ultrasound system (Royal Philips, Amsterdam, Netherlands) equipped with the ElastPQ feature and a 1- to 5-MHz transducer (C5-1). The scan settings for each examination, including gain (50%–70%), scanning depth (14–20 cm), time gain control (homogenous echoes in the near and far field) and mechanical index (~1.2), were optimized for each lesion.

ElastPQ examination

ElastPQ is a shear wave-based elastography technology. It adopts focused ultrasound to induce shear wave propagation inside tissue. Conventional ultrasound is applied to track the shear wave, and tissue elasticity is estimated from the shear wave propagation (Xie et al. 2010). Quantitative measurements within the region