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Quantitative analysis of liver fibrosis in rats with shearwave dispersion ultrasound vibrometry: Comparison with dynamic mechanical analysis

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ABSTRACT

Ultrasonic elastography, a non-invasive technique for assessing the elasticity properties of tissues, has shown promising results for disease diagnosis. However, biological soft tissues are viscoelastic in nature. Shearwave dispersion ultrasound vibrometry (SDUV) can simultaneously measure the elasticity and viscosity of tissue using shear wave propagation speeds at different frequencies. In this paper, the viscoelasticity of rat livers was measured quantitatively by SDUV for normal (stage F0) and fibrotic livers (stage F2). Meanwhile, an independent validation study was presented in which SDUV results were compared with those derived from dynamic mechanical analysis (DMA), which is the only mechanical test that simultaneously assesses the viscoelastic properties of tissue. Shear wave speeds were measured at frequencies of 100, 200, 300 and 400 Hz with SDUV and the storage moduli and loss moduli were measured at the frequency range of 1–40 Hz with DMA. The Voigt viscoelastic model was used in the two methods. The mean elasticity and viscosity obtained by SDUV ranged from 0.84 ± 0.13 kPa (F0) to 1.85 ± 0.30 kPa (F2) and from 1.12 ± 0.11 Pa s (F0) to 1.70 ± 0.31 Pa s (F2), respectively. The mean elasticity and viscosity derived from DMA ranged from 0.62 ± 0.09 kPa (F0) to 1.70 ± 0.84 kPa (F2) and from 3.38 ± 0.32 Pa s (F0) to 4.63 ± 1.30 Pa s (F2), respectively. Both SDUV and DMA demonstrated that the elasticity of rat livers increased from stage F0 to F2, a finding which was consistent with previous literature. However, the elasticity measurements obtained by SDUV had smaller differences than those obtained by DMA, whereas the viscosities obtained by the two methods were obviously different. We suggest that the difference could be related to factors such as tissue microstructure, the frequency range, sample size and the rheological model employed. For future work we propose some improvements in the comparative tests between SDUV and DMA, such as enlarging the harmonic frequency range of the shear wave to highlight the role of viscosity, finding an appropriate rheological model to improve the accuracy of tissue viscoelasticity estimations.

1. Introduction

Liver fibrosis has a high morbidity rate in China and has become one of the most serious public health problems. It results from the process of repairing damaged liver tissue, in which extracellular matrix (ECM) proteins accumulate. The progression of liver fibrosis is complicated and gradual. Currently liver biopsy is the only gold standard for diagnosing of liver fibrosis. Fibrosis staging has been evaluated according to the METAVIR scoring system: F0 represents no fibrosis; F1 represents portal fibrosis without septae; F2 represents portal fibrosis and few septae; F3 represents numerous septae without cirrhosis; and F4 represents cirrhosis [1]. However, liver biopsy can cause serious complications such as bleeding and peritonitis due to its invasive nature. Biomechanical experiments have indicated that elasticity, which is sensitive to physiological changes and the pathological processes of tissues, is one of the most important physical parameters of tissue [2] and has been widely used in liver disease diagnosis. Other physical parameters such as ultrasonic attenuation have recently assessed the status of liver steatosis [3]. Since the early 1990s, studies of non-invasive diagnostic

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methods using the elastic property of tissue have showed very promising results. The goal of elasticity imaging techniques is to assess the stiffness or elastic modulus of tissue, primarily utilizing either of two modalities—ultrasonic elastography [4–15] and magnetic resonance elastography (MRE) [16–21]. Ultrasound elastography has more widespread usage than MRE in clinical applications, due to its lower cost, shorter examination time and lower impact on the human body. Recently, many ultrasound elastographic techniques have appeared, such as quasi-static elastography [9,22–24], transient elastography (TE) [25–28], shear wave elasticity imaging (SWEI) [11,29–32], supersonic shear imaging (SSI) [14,33–36], acoustic radiation force impulse imaging (ARFI) [13,37–43] and shearwave dispersion ultrasound vibrometry (SDUV) [44–50]. Of these techniques, only SDUV includes the measurement of shear viscosity, which may be another indicator of tissue health, while the other modalities only characterize tissue stiffness.

SDUV uses ultrasound acoustic radiation force to characterize the viscoelastic properties of soft tissue. It can quantify shear elasticity and viscosity simultaneously by calculating the shear wave speed at multiple frequencies. Various types of soft tissue, such as liver [49,50], kidney [47], prostate [48], skeletal muscle [46] and artery [51], etc., have been measured by this method. However, no gold standard method has been established for validating SDUV.

It is well known that tissue mechanical properties can also be measured by mechanical tests [52,53]. Some of the mechanical tests have already assessed the accuracy of many ultrasound elastography techniques, such as indentation vs. ARFI on phantoms [39], tensile vs. quasi-static elastography and TE on phantoms [54], indentation vs. SDUV on phantoms [55], dynamic mechanical tests vs. TE on phantoms [56] and porcine livers [57]; but these tests only analyzed and compared the elasticity as measured by these ultrasound methods with the results of mechanical tests. In fact, biological soft tissues are inherently viscoelastic materials [2]. Some studies have reported on the role of tissue viscosity, which has shown potential in grading disease [58–60]. Measurements based on viscoelasticity should be more effective in reflecting the inner mechanical properties of tissue than those based only on elasticity. The dynamic mechanical test is the only mechanical test that can simultaneously evaluate both the elasticity and the viscosity components that comprise the viscoelastic properties of tissue. It should be an ideal test for validating the viscoelastic estimations obtained by SDUV.

In this study, we investigated an independent validation method for SDUV measurements. The viscoelasticity of rat livers at two fibrosis stages was studied quantitatively by SDUV. Dynamic mechanical analysis (DMA) was performed and its measurements were compared with those obtained by SDUV.

2. Methods

2.1. SDUV

Shearwave dispersion ultrasound vibrometry (SDUV) estimates the viscoelastic parameters of a medium by measuring the shear wave speed at multiple frequencies [44]. In SDUV, a localized ultrasound acoustic radiation force is applied to generate harmonic shear waves that propagate outward from the vibration center. Assuming that this is carried out in an isotropic, homogenous and viscoelastic medium, the relationship between the shear wave propagation speed c and the frequency of the shear wave ω in terms of the Voigt model is defined as in [61]

$$c = \sqrt{\frac{2(\mu_1^2 + \omega^2\mu_2^2)}{\rho(\mu_1 + \sqrt{\mu_1^2 + \omega^2\mu_2^2})}} \quad (1)$$

where ρ is the density, μ_1 is the shear elasticity and μ_2 is the shear viscosity of the medium. The shear wave speed at ω is estimated by tracking the phase shift over the distance it propagates [44].

$$c = \omega\frac{\Delta r}{\Delta\varphi} \quad (2)$$

where $\Delta\varphi = \varphi_1 - \varphi_2$ is the phase change over the traveled distance Δr. In this study, dispersion measurements at a fundamental frequency of 100 Hz and its harmonics of 200 Hz, 300 Hz, 400 Hz were substituted into Eq. (1) to solve for the shear elasticity and shear viscosity of the tissue.

2.2. DMA

Dynamic mechanical analysis tests measure the dynamic mechanical behavior of biological tissue. A sinusoidal shear strain $\varepsilon(t) = \varepsilon_0 e^{i\omega t}$ is imposed on the tissue, which induces a sinusoidal shear stress $\sigma(t) = \sigma_0 e^{i(\omega t+\delta)}$ at the same frequency. The ratio of stress to strain amplitude is represented by the complex shear modulus G^* [62]:

$$G^* = \frac{\sigma_0 e^{i(\omega t+\delta)}}{\varepsilon_0 e^{i\omega t}} = \frac{\sigma_0}{\varepsilon_0}(\cos\ \delta + i\sin\delta) = G' + iG'' \quad (3)$$

where ε_0 is the shear strain amplitude, σ_0 is the shear stress amplitude, ω is the angular frequency, δ is a phased-shifted angle. G' is the storage modulus, and G'' is the loss modulus. The complex shear modulus of the Voigt model is given by $G^* = E + i\omega\eta$, where E is the shear elasticity and η is the shear viscosity. Therefore, the equivalence relations to the shear moduli are $G' = E$ and $G'' = \omega\eta$. In this study, the storage moduli and loss moduli of the liver were obtained from the dynamic mechanical tests and fitted with the Voigt model to determine the viscoelastic parameters.

3. Experiments

In total, 35 male SPF Sprague-Dawley rats (provided by Guangdong Medical Laboratory Animal Center, Foshan, Guangdong) weighing 180–270 g were used for SDUV and DMA in vitro experiments. The livers were excised after euthanizing the rats. Fibrosis grading for each rat was obtained by the pathological section and Masson Trichrome stain. The control group (stage F0) contained 12 rats and the model group (stage F2) contained 23 rats. Six in F0 and 15 in F2 were used for the SDUV experiments, the rest were used for the DMA tests. The largest lobe from each liver, usually about 4–6.3 cm length, was chosen as the experimental sample in both methods.

We were unable to use the same liver sample in the SDUV and DMA tests because of conflicts between the requirements of the two systems. In the SDUV, we chose the largest liver lobe and embedded it in the fabricated gelatin solution (gelatin from porcine skin, Sigma–Aldrich, St. Louis, MO) in a container ($15 \times 15 \times 10\,cm^3$) in order to avoid having the liver sample float in the water tank. After several hours the solution would cool down to room temperature ($23 \pm 1\,^\circ C$) and the liver would integrate with the gelatin. Then we took out the liver phantom from the container and turned it upside down in the water tank (because the liver sank to the bottom). After conducting the SDUV experiments, we were unable to separate the liver from the gelatin. Therefore, if the liver had been used in the subsequent DMA tests, it would cause a measurement error. On the other hand, if we had performed the DMA tests first, we would have had to cut the largest liver lobe into slices because the DMA was a rheometer with a 25 mm-diameter parallel plates configuration. The small size of these slices restricted the focal area of the two transducers in the SDUV. Therefore, because of the limits of the experimental conditions, we divided the rats into separate parts for the two methods.

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