RESEARCH ARTICLE

Transrectal Real-time Tissue Elastography - An Effective Way to Distinguish Benign and Malignant Prostate Tumors

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Abstract

Background: To investigate the relationship between extracellular matrix parameters and texture of prostatic lesions evaluated by transrectal real-time tissue elastography (TRTE). Methods: 120 patients suspicious for prostate cancer underwent TRTE. Targeted biopsies were carried out after 12-core systematic biopsy. Epithelia were stained with hematoxylin-eosin, and Victoria blue and Ponceau S were used to stain elastic-collagen fibers, and picric acid-sirius red for visualization of collagen type I (Col1) and III (Col3). Smooth muscles were visualized by immunohistochemistry. All image analyses were performed in a blind manner using Image Pro Plus 6.0, and the area ratios of epithelium, elastic fibers, collagen fibers and Col1/Col3 were determined. Results: 42 patients with typical elastograms were included in the final data analysis. Significant differences were detected between the benign and malignant groups in the area ratios of epithelium (P = 0.01), smooth muscles and Col1/Col3 (P = 0.04, P = 0.02, respectively). There were no significant differences in the area ratios of epithelium, smooth muscle and elastic fibers between the stiff and soft lesion groups. The area ratio of Col1 was (0.05±0.03) in the stiff group, and (0.02±0.01) in the soft group (P= 0.00). However, the area ratio of Col3 was (0.03±0.02) in the stiff group, and (0.05±0.04) in the soft group (P = 0.16). Col1/Col3 in the stiff group (1.99±1.59) was greater than in the soft group (0.71±0.64) (P = 0.01). Conclusions: Tissue hardness of prostatic tumors was mainly dependent on the Col1 content, Col1/Col3 being higher in malignant than in benign lesions, so the prostate tissue texture can be used as a target for distinguishing between the two with TRTE.

Keywords: Elastography - ultrasound - prostate cancer - benign/malignant - differential diagnosis - collagen

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Introduction

The elasticity or stiffness of biologic tissues depends on their molecular composition, and on the microscopic and macroscopic structural organization of these molecules. Although there is little difference in the elasticity between the different anatomies in normal tissues (Greenleaf et al., 2003), significant differences exist in the bioelasticity of normal and diseased tissues (Agliamov et al., 2000). Previous studies showed that a change in tissue elasticity can occur before the observation of morphological changes in the tissue (Gao et al., 1997; Sumura et al., 2007). Therefore, manual digital rectal examination (DRE) has been used as a means of clinical diagnosis for many centuries.

Imaging modalities such as conventional ultrasound (US), magnetic resonance imaging (MRI) and computer tomography (CT) do not provide information about tissue texture. Ultrasound elastography is a unique method which combines the advantages of DRE and ultrasound for the assessment of the bioelasticity distribution in vivo by displaying strain images of tissues. Studies have shown that elastography improves the diagnostic effectiveness for prostate cancer (PCa) by compensating for the limitations of DRE, such as the lesion depth and the physician's experience (Miyanaga et al., 2006; Pallwein et al., 2007; Belbase et al., 2013). This technique has been applied widely in the diagnosis of many kinds of tumors. To date, there are several reports of the use of transrectal real-time tissue elastography (TRTE) in the diagnosis of PCa and in the treatment evaluation and follow-up of the prostatic lesions (Souchon et al., 2003; Fahey et al., 2005; Aigner et al., 2010; Zhang et al., 2012; Zhang et al., 2012). However, most of these studies have focused on the differential diagnosis of prostatic lesions, and reports on the pathologic conditions corresponding to the elasotgraphy of stiff and soft lesions have not been investigated.

Although malignant tumors exhibit stiff properties compared with normal tissues and benign tumors, the reasons for this difference remain to be elucidated. It has been speculated that tissue stiffness is the result of the increased cell density in malignant tissues (Krouskop et al., 1998; Pallwein et al., 2008) although others have proposed that the mechanical properties of biological tissues depend on their constituent macromolecules (parenchyma, fat, collagen, etc.) and on their structural organization (Gheorghe et al., 2008). The prostate

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structure is predominantly formed by two components: gland and stroma. The extracellular matrix (ECM) component comprises smooth muscle and fibrous components (collagen fibrillar system, microfibril elastin system) (Ushiki et al., 2008). The aim of this study was to analyze the content of epithelia, smooth muscle, collagen fibers and elastic fibers in the prostatic lesions, and to investigate the relationship between the tissue texture evaluated by elastography and these tissue components. We hope to find a new target to distinguish benign and malignant prostate tumors at first step using transrectal real-time tissue elastography (TRTE).

Materials and Methods

Patients

Between December 2009 and February 2010, a total of 120 patients with suspected PCa lesions underwent prostate biopsy. They were scheduled for prostate biopsies because of serum PSA level (>4 ng/ml), palpable nodular lesions in DRE, hypoechoic lesions on transrectal ultrasound scan (TRUS) or abnormal MRI findings (low-intensity lesions on T2-weighted images).

All patients underwent TRTE examinations and prostate biopsy. The study was approved by the local Ethics Committee. Before biopsy, the risks and benefits of the biopsy procedures were explained to each patient, and written informed consent was obtained from every patient at enrollment. Histopathologic findings of the typical softness (no blue was found in the lesion) and stiffness (the whole lesion was blue) were analyzed, and 42 patients were included in the final data analysis (19 soft cases and 23 stiff ones). The average age of patients was 66.56±8.99 years (range, 52-84 years). Exclusion criteria were active urinary tract infection and preoperative endocrine treatment (Aigner et al., 2010) or previous transurethral surgery.

Imaging technique

Real-time tissue elastography was conducted using the HI VISION 900 ultrasound system (Hitachi Medical, Tokyo, Japan) with the EUP-V53W probe set at a frequency of 4-9MHz. All patients were scanned in the left decubitus position with buttocks located at the edge of the examination bed and knees bent toward the chest. First, the prostate was examined by TRUS. Once lesions were detected, their position, size, boundaries and internal echoes were recorded. Second, the real-time tissue elastography mode was switched on and the elastogram was shown on the monitor together with the grey-scale ultrasound images. In general, the sampling box was set such that it was large enough to contain the total image.

TRTE was performed by slight compression and decompression of the prostate, which was induced manually by a physician using the TRUS probe. The applied force was adjusted according to the visual indicator for compression. The TRTE images were obtained transversely from the base to the apex of the prostate in sequence, and were recorded on the internal hard disk of the ultrasound equipment. The stiffness of lesion was displayed from red (soft) to green (intermediate) and blue (hard). The color-coding is standardized and the same color display was used for all patients.

Based on the criterion proposed by Konig et al. (2005), the blue-colored areas, which had a diameter of at least 5 mm and which were reproducible (after tilting of the US probe) on the elastogram, were regarded as stiff region. TRTE-targeted biopsy towards the stiff regions and/or soft regions of the prostate peripheral zone was performed transrectally for each patient using an 18-gauge biopsy needle (Biopty; Bard, Covington, GA, USA) powered by an automatic biopsy device. The resulting biopsy specimens were collected, marked and fixed in 10% formaldehyde at room temperature. The tissues were then embedded in paraffin, and 4 serial sections with the thickness of 4 μm were obtained. Sections obtained from every specimen were stained with hematoxylin & eosin (H&E) for visualization of epithelia, double staining using Victoria blue & Ponceau S for visualization of elastic-collagen fibers, picric acid-sirius red for visualization of collagen type I (Col1) and III (Col3). Immunohistochemistry using UltraSensitiveTM S-*P* Kit (KIT-9710, purchase from Maixin-bio) was performed for smooth muscle. A mouse monoclonal anti-Ki-67 antibody (Santa Cruz, USA, 1: 100) was used to stain Ki-67 protein, and the second antibody used Biotinylated anti-mouse immunoglobulin. Tan nuclear stainning was considered to be positive.

Images were visualized on a polarized microscope (6000B, LEICA DM, Germany) with the Image Acquisition Card (LAS V4.0). Specimens stained with H&E were analyzed by a specialized prostate pathologist with 20 years of experience. Six fields of each section were randomly selected in the form of "S" for evaluation (200× magnification).

Pathology analysis

All image analysis was performed in a blind manner using Image Pro Plus (version 6.0, Media Cybernatics, USA). The area ratio of epithelia, elastic fibers, collagen fibers, smooth muscle and Col1 and Col3 in each section was calculated respectively.

Statistical analysis

Data analysis was performed using the SPSS software package, version 11.5 for Windows (SPSS Inc., Chicago, IL). Quantitative data conforming to the normal distribution and with equal variance were analyzed by t-test. *P* values <0.05 were considered to be statistically significant.

Results

Area ratio of epithelia and stromal fibers in BPH and PCa groups

Among the 42 patients included in this study, 20 were diagnosed with benign prostatic hyperplasia (BPH), while the remaining 22 cases were diagnosed with PCa (Gleason score range, 6-9).

The area ratio of epithelia, elastic fibers, collagen fibers and smooth muscles of the prostatic ECM are shown in Table 1. There was a significant difference in the area

Table 1. The Area Ratio of Epithelia, Fibers and Smooth Muscle of the Stroma in Benign and Malignant Groups

Group	Elastic fibers	Collagen fibers	Col 1	Col 3	Col1/Col3 ratios	Smooth muscle
Benign	0.03±0.03	0.09±0.05	0.03±0.02	0.05±0.03	0.83±1.12	0.15±0.23
Malignant	0.02±0.02	0.11±0.10	0.04±0.03	0.03±0.02	1.94±1.57	0.01±0.02
p	0.11	0.49	0.07	0.08	0.02	0.04

Table 2. The Area Ratio of Elastic Fibers, Collagen Fibers and Smooth Muscles of the Prostate Extracellular Matrix in Soft and Stiff Groups

Group	Elastic fibers	Collagen fibers	Col 1	Col 3	Col1/Col3 ratios	Smooth muscle
Soft	0.04±0.02	0.09±0.04	0.02±0.01	0.05±0.04	0.71±0.64	0.07±0.07
Stiff	0.03±0.03	0.09± 0.04	0.05±0.03	0.03±0.02	1.99±1.59	0.09±0.21
p	0.77	0.94	0.002	0.16	0.007	0.82

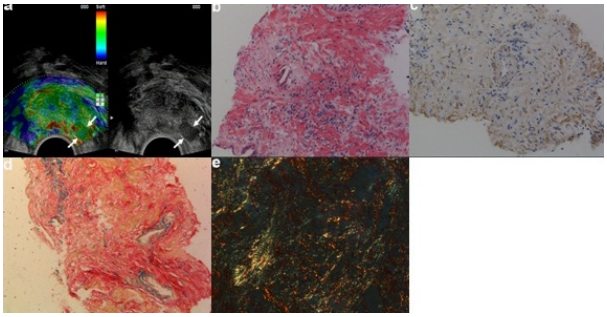

Figure 1. Elastography of Benign Prostatic Hyperplasia (Soft Lesion) (a) A hypoechoic lesion with clear boundary was presented in the left side of the prostate by transrectal ultrasound scan (right image, white arrow), and a local green region (soft) was displayed in the lesion by transrectal real-time tissue elastography (left image, white arrow). (b) Pathological analysis showed the lesion was benign hyperplasia. (c) Immunohistochemical analysis showed that the smooth muscle (tan region, 0.13) content in the extracellular stroma of the prostate was high. (d) Double staining showed that the area ratio of elastic fibers (blue region, 0.01) was less than that of the total collagen fibers (red region, 0.11). (e) Picric acid-sirius red staining showed that the area ratio of Col3 (green region, 0.09) was greater than that of Col1 (red region, 0.04)

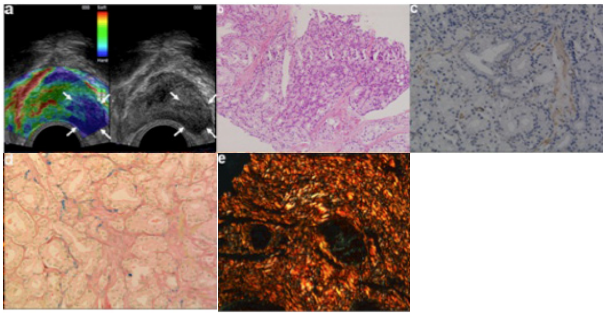

Figure 2. Elastography of Prostate Cancer (Stiff Lesion). (a) A hypoechoic lesion without clear boundary presented on the left side of the prostate by transrectal ultrasound scan (right image, white arrow), and a local blue region (stiff) displayed in the lesion by transrectal real-time tissue elastography (left image, white arrow). (b) Pathological analysis showed the lesion was prostate cancer with a Gleason score of 7. (c) Immunohistochemical analysis showed that the smooth muscle (tan region, 0.03) content in the stroma of prostate was low. (d) Double staining showed sparse distribution of elastic fibers (blue region) in the extracellular stroma, with an area ratio (0.01)that was less than that in the collagen fibers (red region, 0.06). (e) Picric acid-sirius red staining showed that the area ratio of Col1 (red region, 0.22) was greater than that of Col3 (green region, 0.02)

ratio of the epithelia (P = 0.01) between the BPH and PCa groups, and small but significant difference in the area ratio of smooth muscles between the two groups (P = 0.04). No significant differences were detected in other parameters between the benign and malignant groups (P > 0.05). Although there were no significant differences in area ratios of Col1 and Col3 between the two groups (P > 0.05), a significant difference was detected in the Col1/Col3 ratios between the BPH (0.83±1.12) and PCa (1.94±1.57) groups (P = 0.02).

Area ratio of epithelia and stromal fibers in soft and stiff groups

Prostatic epithelia and lesion texture: TRTE examination of the 42 patients revealed 19 cases of soft tissue (Figure 1a, b) (15BPH, 4 PCa) and 23 cases of stiff tissue (Figure 2a, b) (5 BPH, 18 PCa). The diagnostic sensitivity, specificity, and accuracy of PCa were 78.3%, 78.95%, and78.6% by TRTE. There was no significant difference in the mean epithelial area ratio in the H&E stained sections between the soft and stiff tissue cases (0.04±0.03 vs. 0.09±0.09, respectively; P = 0.13).

Prostatic extracellular matrix and lesion texture: The area ratio of elastic fibers, collagen fibers and smooth muscles of the prostatic ECM in the soft and stiff groups are shown in Table 2. Immunohistochemical analysis showed that there was no difference in the area ratios of the smooth muscle between the soft and stiff groups (P = 0.82) (Figure 1c, 2c). There was no significant difference in the area ratios of the elastic fibers and collagen fibers in sections between the soft and stiff groups (P = 0.77, 0.94, respectively) (Figure 1d, 2d).

Picric acid-sirius red staining showed that there were significant differences in the area ratios of Col1 between the stiff and soft groups (0.05±0.03 vs. 0.02±0.01, respectively; P = 0.00). However, no significant difference was detected in the area ratios of Col3 between the stiff and soft groups (0.03±0.02 vs. 0.05±0.04, respectively; P = 0.16) (Figure 1e, 2e).

Discussion

PCa is the second leading cause of cancer death in the Western world (Peters et al., 2005), and its incidence is also increasing in the Asia- Pacific region year by year (Moore, 2013). Early detection can obviously improve

chances of long-term survival. Since 1998, when Krouskop et al. confirmed a significant difference in the mechanical behavior between normal and cancerous prostate tissues, elastography has been used as a new imaging technique for the diagnosis of PCa (Hoyta et al., 2008). Elasticity images obtained by quantitative sonoelastography are consistent with the results of mechanical and histological evaluation. For normal and cancerous prostate samples, the average magnitudes of the complex Young's moduli (|E*|) were 15.9±5.9 kPa and 40.4±15.7 kPa at 150 Hz, respectively, giving an elastic contrast of 2.6:1. There were significant differences between the stiffness of normal and cancerous prostate tissues in the same gland ($P < 0.01$). Previous studies have shown that elastography is a valuable technique for the diagnosis of PCa with high diagnostic accuracy (84.1-91%) (Konig et al., 2005; Pallwein et al., 2007). We have accumulated some experiences in the operation and image identification, and have developed diagnostic criteria of the elastograms (Zhang et al., 2012). Therefore, elastography can be used to reflect the tissue texture well. Although, subsequent clinical diagnosis reveals that some patients are still misdiagnosed using this technique. For example, in the study by Pallwein et al. (2007), 51.6% of lesions with inflammation were misdiagnosed as PCa, largely due to a lack of understanding of the correlation between tissue pathology and elastogram results. Our previous study showed that the detection rate of PCa using TRTE-targeted biopsy (75.8%) was significantly higher than that of systematic 12-core biopsy plus TRUS-targeted biopsy (14.5%) (P =0.00), and can reduce the number of cores effectively (Zhang et al., 2012), so TRTE-targeted biopsy was used to get the tissues in this study.

Epithelial-stromal interactions in the prostate indicate that the response of the stroma reaction to invading cancer is characterized by elevated collagen deposition, which can lead to increased stiffness of the cancerous tissue. Furthermore, a similar stromal response was observed in the growth of hyperplastic nodules, although most of the benign nodules were not stiff. Analysis of the area ratio of the epithelia and components of the stroma showed that smooth muscle was the main component in the stroma of benign lesions, and were significantly destroyed in the malignant lesions with concomitant fiber hyperplasia. These observations are consistent with a previous report (Birbach et al., 2011). Moreover, there was a significant difference in the epithelial area ratio between the BPH and PCa groups.

The correlation between fiber hyperplasia and/or the number of epithelia and differences in stiffness between benign and malignant nodules is unclear. In this study, no differences were detected in the collagen fibers, which provide biomechanical rigidity, between the benign and malignant groups. However, double-staining showed the presence of collagen type I, II, III and IV. It is well-known that the distribution of distinct collagen fibers differs throughout the human body, each with different mechanical properties. Collagen type I, II and III occurs in the ECM, while type IV exists in the basement membrane of tissue. Collagen type II occurs predominantly in cartilage and vitreous; therefore, the type II collagen content in the prostate is low (Tao et al., 1998). Analysis of the prostatic content of Col1 and Col3 showed a difference in the area ratio of Col1 /Col3 between the benign and malignant groups. In order to investigate the relationship between tissue texture and Col1 or Col3 content the prostatic lesions were divided into two groups (soft vs. stiff) according to the results of elastograms. There was a significant difference in the contents of Col1 but not Col3 between the soft and stiff groups. Compared with other types of collagen fibers, increased type I content corresponds to more rigid and less compressible tissue properties (Wenger et al., 2007; Shoulders et al., 2009), and is distributed in the bones and tendons which form the rigid structure of the human body. In areas of high Col1 content are the arrangement of collagen fibers was thick while the arrangement of collagen fibers was loose in areas rich in Col3 (Zhi et al., 1995). Col3 is distributed in the tissues with high elasticity, such as blood vessels and skin. Therefore, the results of this study indicate a good correlation between the content of Col1 and tissue texture. Furthermore, there was no difference of the epithelial content between the soft and the stiff groups, which suggested that the increased number of epithelia did not account for lesion stiffness. In this study, there was no difference in the elastic fibers of the stroma between the soft and stiff groups. A previous study (Zhang et al., 2003) clearly showed that the elastic fibers were distributed in the transitional zone of the normal prostate. The specimens analyzed in our study were biopsied from the peripheral zone of the prostate and therefore, the small proportion of elastic fibers did not affect the tissue texture. Furthermore, although the smooth muscle content in the PCa group was lower than that in the BPH group, there was no significant difference between the soft and stiff groups, thus suggesting that smooth muscle content is not the main factor that influences tissue texture.

In conclusion, tissue hardness of prostatic lesions were not found to correlate with the number of epithelia, but was mainly dependent on the Col1 content with increased tissue stiffness increasing with the Col1 content. Col1/Col3 in malignant lesions was greater than that in the benign lesions, which in combination with the serious destruction of smooth muscle observed in malignant lesions may account for the increased tissue rigidity in cancerous tissues compared with benign tissues. The Tissue texture would become a promising indicator for diagnosing of PCa.

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