Mechanical Property Characterization of Prostate Cancer Using a Minimally Motorized Indenter in an Ex Vivo Indentation Experiment

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OBJECTIVES
To measure the mechanical property of prostatic tissues using a minimally motorized indenter and to determine whether measurable differences in mechanical property exist between cancerous and noncancerous tissues in an ex vivo experiment.

METHODS
A total of 552 sites from 46 prostate specimens taken during radical prostatectomy underwent an indentation experiment with a minimally motorized indenter, and the elastic modulus (Young's modulus) of the tissue was estimated.

RESULTS
The mean elastic modulus of the regions containing cancer and noncancer was 24.1 ± 14.5 and 17.0 ± 9.0 kPa, respectively. In the noncancerous regions, the prostate was separated into 5 parts according to the post hoc test for comparing the elastic modulus between the 2 groups: part 1, lateral apex; part 2, medial apex; part 3, lateral-mid; part 4, lateral base; and part 5, medial-mid and medial base. In the regions containing cancer tissue, the prostate was also separated into 5 parts: part 1, lateral apex and medial apex; part 2, lateral-mid; part 3, lateral base; part 4, medial base; and part 5, medial-mid. The elastic modulus was greater in the tissue with a Gleason score of 8 than in the other tissue. The elastic modulus was significantly greater in the tissue with a tumor volume >5 cm³ than in the other tissue.

CONCLUSIONS
We determined the elastic moduli of prostatic tissue as a quantitative and objective parameter according to the regions of the prostate, the presence of cancerous tissue, the tumor volume, and the Gleason score.

Prostate cancer is the most prevalent cancer among men in the United States and European countries and is the second-leading cause of death of men in the United States.1,2 The diagnosis of prostate cancer has been done using screening tests for most cases. Although the optimal screening strategy has not yet been defined, serum prostate-specific antigen (PSA) determination and digital rectal examination (DRE) are the most commonly used screening tests. Most cases of prostate cancer arise in a posterior location, and cancer tissue is grittier and firmer than normal tissue. Thus, prostate cancer is often palpable on the DRE.3 Thus, a careful DRE can detect early or small prostate carcinoma. Although many cases of prostate cancer have frequently been detected before they could be palpated on DRE since the introduction of PSA, the use of DRE is still important because abnormal findings on the DRE are an independent predictor of prostate cancer, especially high-grade cancer,4,5 and are also used in additional prediction models for clinically significant tumors.6 Moreover, the positive predictive value of abnormal DRE findings has been ≤33% even in the case of men with a PSA level of 4 ng/mL.7 However, the results of DRE are subjective and dependent on physician experience. These are the limitations of the DRE as a diagnostic tool or clinical parameter. Therefore, objective and quantitative parameters related to the prostatic tissue during the DRE might be useful to physicians for assessing the condition of the prostatic tissues. One possible approach to obtain objective parameters is the characterization of the mechanical property of the biologic tissue. To obtain the mechanical property of tissue, several methods have recently been developed. Indentation is one of the methods used to measure the mechanical property of tissue by applying a dynamic deformation and concurrently observing the reaction forces of the
tissue. In previous studies, the experiments that measured the mechanical properties of prostatic tissue were useful in the assessment of prostate conditions, both benign and malignant. However, most studies were performed in vitro. Thus, the aims of the present study were to measure the elastic modulus (E) as a mechanical property of the prostatic tissue using a minimally motorized indenter and to determine whether differences in the mechanical properties were present between the cancerous and non-cancerous tissue in an ex vivo experiment.

MATERIAL AND METHODS

Prostate specimens were obtained from 46 patients who had undergone radical prostatectomy at Severance Hospital, Yonsei University in Korea from April 2009 to July 2009. Patients who had undergone preoperative hormonal therapy, radiotherapy, or prostate-related surgery were excluded from the present study. Patients with clinically insignificant small cancer (<0.5 cm³) were also excluded from the analysis. A total of 40 prostate specimens from 46 patients were eligible for analysis. The mean patient age was 63.0 years (range 44-69), and the mean PSA level was 12.9 ng/mL (range 2.8-120.9). The mean preoperative prostate volume was 41.5 cm³ (range 18.27-115.9). The pathologic cancer stage was T1c in 31 patients, T2a in 6 patients, T2b in 5 patients, T2c in 1 patient, T3a in 1 patient, and T3b in 2 patients. All patients provided written informed consent, and the institutional review board approved the study. The elastic modulus of the tissues was measured within 30 minutes after removal in the operating room using a minimally motorized indenter (Fig. 1). The minimally motorized indenter consisted of a micrometer (Maxon Precision Motors, Sachsin, Switzerland), a linear position sensor (magnetoresistive sensor, pieotech, Seoul, Korea), a force transducer (Kistler Instrument, Munich, Germany), and a hemisphere tip and cylindrical body (2-mm diameter) probe (Misumi, Tokyo, Japan). This system was designed to have a maximal outer diameter of 8 mm and a length of 150 mm. The indenter was controlled by a micro-DC motor, and the position was sensed with an integrated magnetoresistive sensor. To validate the uniformity of our system, we performed motion calibration of the system using a laser motion sensor. During the motion calibration, the indenter was manipulated within a range of 8 mm, with <10-μm motion errors. The reaction force at the probe tip was measured with the force transducer (resolution 1 mN) and recorded using the data acquisition system (NI PCI-6221, National Instruments, Austin, TX). In addition, we performed indentation tests to validate the system performance for measuring tissue behavior using the tissue phantom (RTV silicone gel 6166, GE Silicones, Wilton, CT). The force response was measured against a 7-mm indentation loading of a 3-mm diameter indenter. Young’s modulus was estimated using the Hertz-Sneddon equation (Eq. 1) as 4.97 kPa, similar to the results of a previous study. The investigator who performed the mechanical testing was unaware of the clinical data. This test consisted of the following steps. First, the indenter was brought into contact with the posterior surface of the prostate and induced a 3-mm depth indentation (position control) at a speed of 1 mm/s in the tissue to make the deformation. When the deformation was induced in the tissue, the reaction forces occurred and were measured with the force transducer. Second, data on the reaction forces and deformation were acquired using the data acquisition system. After completion of the indentation, the indenter was moved backward from the indented surface and repositioned to indent at other sites. For each prostate specimen, the indentation experiment was performed at 12 sites across the posterior surface of the prostate. These 12 sites were similar to those of double-sextant needle biopsies, including the lateral apex (LA), lateral-mid (LM), lateral base (LB), medial apex (MA), medial-mid (MM), and medial base (MB). A total of 552 indentations were performed on the 46 specimens. The mechanical properties of the biologic tissues are presented as the elastic modulus (E). If the indenter contacted perfectly with the tissue, the assumed semi-infinite elastic body, the Hertz-Sneddon equation (Eq. 1) was applied to estimate the elastic modulus of the tissue from the experimental results.

\[
E = \frac{3}{4} \frac{(1 - \nu^2) f_z}{\sqrt{R \cdot \delta}}
\]

where E, f_z, R, and \( \delta \) are the elastic modulus, reaction force, indenter radius (2 mm), and indentation depth (3 mm), respectively. The Poisson’s ratio \( \nu \) was 0.499. The elastic modulus of the tissues was estimated from the experimental results using Eq. 1. After the investigator completed the mechanical testing, the tissue samples were sent to the pathology department for histologic examination. All specimens were fixed in formalin and embedded in paraffin. The specimens were then stained with hematoxylin-eosin and examined with an optical microscope. The specimens were analyzed by a single pathologist who...
was unaware of the results of the mechanical testing. The location and size of each tumor lesion were documented for all sections. The elastic moduli of the prostatic tissues containing cancer and the noncancerous tissues is presented as the mean ± SD. The 2-sample, independent t test was used to assess the differences between the elastic moduli of the cancerous and noncancerous tissue. The differences between the groups according to the region of the prostate specimen, Gleason score, and tumor volume were assessed by analysis of variance, followed by the post hoc test to analyze the pairwise differences. All statistical tests were 2-sided (α level 0.05). The Statistical Package for Social Sciences, version 12.0 (SPSS, Chicago, IL) was used to perform the statistical analyses.

RESULTS
Overall, mechanical data (elastic modulus) from 552 regions were obtained using the indentation experiment, and the results from the mechanical analysis were compared with the tumor location, tumor volume, and Gleason score, as documented by the pathologist. However, the data from 72 regions from 6 patients were excluded from the analysis because of mechanical or clinical data corresponding to the exclusion criteria. Of the 480 regions from 40 prostate specimens, 206 revealed cancerous tissue on histopathologic examination. The tumors were distributed most frequently at the base of the prostate (36.4%), followed by the mid-gland (34.5%) and the apex of the prostate (29.1%) without statistically significant differences (P = .215). The mean elastic modulus of the regions containing cancerous and noncancerous tissue was 24.1 ± 14.5 and 17.0 ± 9.0 kPa at a 3-mm indentation (P = .000), respectively. This result showed that the region containing cancerous tissue increased the elastic modulus. The elastic modulus according to the location of prostate cancer tissue is listed in Table 1. In the noncancerous regions, the MB demonstrated the greatest elastic modulus (25.4 kPa), followed by the MM (21.7 kPa), LB (17.7 kPa), LM (16.4 kPa), MA (12.9 kPa), and LA (11.0 kPa). In the regions containing cancerous tissue, the MM demonstrated the greatest elastic modulus (32.9 kPa), followed by the MB (30.8 kPa), LB (24.3 kPa), LM (21.5 kPa), LA (15.2 kPa) and MA (14.6 kPa). Using the results of the elastic modulus determination and the post hoc test, we separated the prostate into 5 parts. In the regions containing noncancerous tissue, the prostate was separated into 5 parts: part 1, LA; part 2, MA; part 3, LM; part 4, LB; and part 5, MM and MB. In the regions containing cancerous tissue, the prostate was separated into 5 parts: part 1, LA plus MA; part 2, LM; part 3, LB; part 4, MB; and part 5, MM (Fig. 2). In the regions containing cancerous tissue, significant differences were found in the elastic modulus between the 2

Table 1. Elastic modulus according to prostate region

<table>
<thead>
<tr>
<th>Prostate Region</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>95% CI</th>
<th>Post Hoc Test (Tukey B*; Subset for α = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Noncancerous tissue (P = .000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>52</td>
<td>11.04</td>
<td>5.23</td>
<td>9.58-12.50</td>
<td>11.04</td>
</tr>
<tr>
<td>MA</td>
<td>48</td>
<td>12.85</td>
<td>6.37</td>
<td>11.00-14.70</td>
<td>12.85</td>
</tr>
<tr>
<td>LB</td>
<td>48</td>
<td>17.75</td>
<td>6.82</td>
<td>15.77-19.73</td>
<td>17.75</td>
</tr>
<tr>
<td>MB</td>
<td>37</td>
<td>25.43</td>
<td>10.00</td>
<td>22.10-28.77</td>
<td>25.43</td>
</tr>
<tr>
<td>Cancerous tissue (P = .000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>28</td>
<td>15.18</td>
<td>11.41</td>
<td>10.76-19.60</td>
<td>15.18</td>
</tr>
<tr>
<td>LB</td>
<td>32</td>
<td>24.31</td>
<td>11.97</td>
<td>20.00-28.63</td>
<td>24.31</td>
</tr>
<tr>
<td>MB</td>
<td>43</td>
<td>30.81</td>
<td>14.98</td>
<td>26.20-35.43</td>
<td>30.81</td>
</tr>
<tr>
<td>MM</td>
<td>39</td>
<td>32.87</td>
<td>15.51</td>
<td>27.85-37.90</td>
<td>32.87</td>
</tr>
</tbody>
</table>

CI, confidence interval; LA, lateral apex; MA, medial apex; LM, lateral-mid; MM, medial-mid; LB, lateral base; MM, medial-mid; MB, medial base.

Type I error levels not guaranteed.
Mean for groups in homogeneous subsets displayed.
* Group sizes were unequal; harmonic mean of group size used (harmonic mean sample size 45.063).
groups when stratified by the Gleason score. The elastic modulus was greatest in those with a Gleason score of 8, followed by those with a Gleason score of 6, 7, and 9 (P = .000; Table 2). We also compared the elastic modulus according to the tumor volume. When all cases were divided into 3 subgroups (0.5-1, 1-5, and >5 cm³) according to tumor volume, significant differences were found in the elasticity among the 3 subgroups. In particular, the elastic modulus was significantly greater in those with a tumor volume >5 mL than those in the other subgroups (Table 2). We also evaluated the correlation between the prostate weight and the elastic modulus of the specimen. In the regions without cancerous tissue, no significant correlation was found between the prostate weight and elastic modulus, except for MM, which showed a significant correlation in linear regression analysis (R² = 0.196, P = .004). In the regions containing cancerous tissue, the elastic modulus showed no significant correlation with the prostate weight.

**COMMENT**

Changes in the mechanical properties reflect the changes in the integrity of biologic tissue because pathologic changes alter the properties of tissue owing to transformation of the integrity of cells or intercellular matrices. Malignancy can also alter tissue integrity and affect the properties. To measure the properties of biologic tissue, many researchers have performed experiments using numerous techniques, including indentation, aspiration, shear strain, and compressive pressure. In general, they applied specific mechanical loadings to the surface and recorded the tissue responses that reflected the mechanical properties. Prostate adenocarcinoma has a greater cellular density than the surrounding normal tissue. The pathologic findings of prostate adenocarcinoma have revealed that tumor cells have well-defined glandular patterns and are smaller and more crowded than normal glands, resulting in an increase in cellular density. These changes increase the mechanical properties of the prostate. Prostate adenocarcinoma also leads to subsequent changes in the surrounding normal tissue. Normal stromal tissue produces collagen to repair the damage resulting from cancer cell invasion. These changes result in excessive collagen deposits in the normal tissue surrounding the cancerous tissue. Additionally, the cancer tissue increases the induration from the changes and becomes harder than normal tissue on DRE. Furthermore, the amount of accumulated collagen correlated with tumor aggressiveness. These results are in accordance with the clinical features of DRE-positive tissue. Prostate cancer detected by DRE alone shows aggressiveness, and an abnormal DRE finding is an independent predictor of high-grade prostate cancer. However, the subjective findings and low accuracy of DRE have limited its wide use in clinical practice. Several studies of the mechanical properties of the prostate compared with the histologic findings have been conducted. Krouskop et al investigated the properties of prostatic tissue obtained from a compression loading experiment and showed that the elasticity of prostate cancer tissue was greater than that of normal tissue. Jalkanen et al have demonstrated that cancer tissue increased in stiffness according to the tissue morphology in the results of an experiment with a piezoelectric resonance sensor. Other previous studies using dynamic indentation have also shown that the properties of prostatic tissue correlate with tissue integrity and the presence of prostate cancer, which consists of smaller and tightly packed acini. However, most previous studies were conducted in vitro. Recently, ultrasound real-time elastography has been clinically applied to measure and visualize structural differences, such as tissue elasticity. However, the visualized information of elasticity marked using color is not a quantitative and objective parameter, because the information varies according to the degree of the free-hand compression of the probe. In our study, we acquired quantitative data on the mean elastic modulus of the regions containing cancerous and noncancerous prostatic tissues in ex vivo experiments. These results are consistent with those of previous studies, indicating that pros-
tate cancer tissue is usually stiffer than the surrounding normal tissue. However, the results of the present study have limitations in the direct application to an in vivo trial owing to the wide range of elastic modulus values. This has been attributed to the innate mechanical characteristics of the prostate reflected by the histopathologic conditions, such as tumor site, tumor volume, and Gleason score. Therefore, we should consider these differences when determining the cutoff value for discriminating between cancerous and noncancerous tissue for an in vivo trial. The second limitation was that uncertainty was present in exactly matching the indentation place with the center of the tumor location on microscopic examination because the ex vivo mechanical measurements were performed blindly without any information on the tumor location. Therefore, we classified the tumor location according to the different elastic moduli. The modulus of prostate tissue was present in exactly matching the indentation place in cancerous and noncancerous tissue, the prostate was separated into 5 parts according to the different elastic moduli. The modulus of the prostate increased if it contained cancerous tissue, the tumor volume was $>5$ cm$^3$, or the Gleason score was 8. However, additional studies are needed to assess the usefulness of the mechanical property (elastic modulus) as a clinical parameter. In addition, we will evaluate whether our objective and quantitative data on elasticity could be a marker that discriminates cancerous tissue from normal tissue.

CONCLUSIONS

We have developed an minimally motorized indenter to measure the mechanical properties of biologic tissue and also assessed the properties of prostate cancer. We acquired the elastic moduli of the prostatic tissue as a quantitative and objective parameter according to the prostate regions, presence of cancerous tissue, tumor volume, and Gleason score. In both cancerous and noncancerous tissue, the prostate was separated into 5 parts according to the different elastic moduli. The modulus of the prostate increased if it contained cancerous tissue, the tumor volume was $>5$ cm$^3$, or the Gleason score was 8. However, additional studies are needed to assess the usefulness of the mechanical property (elastic modulus) as a clinical parameter. In addition, we will evaluate whether our objective and quantitative data on elasticity could be a marker that discriminates cancerous tissue from normal tissue.

References