Ultrasonic attenuation estimation of the pregnant cervix: a preliminary report

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KEYWORDS: attenuation; cervical length; cervical ripening; detection of cervical ripening; preterm birth

ABSTRACT

Objective Estimates of ultrasonic attenuation (the loss of energy as an ultrasonic wave propagates through tissue) have been used to evaluate the structure and function of tissues in health and disease. The purpose of this research was to develop a method to estimate ultrasonic cervical attenuation during human pregnancy using a clinical ultrasound system.

Methods Forty women underwent a cervical scan once during pregnancy with the Zonare z.one clinical ultrasound system using a 4–9-MHz endovaginal transducer. This ultrasound system provides access to radiofrequency (RF) image data for processing and analysis. In addition, a scan of a tissue-mimicking phantom with a known attenuation coefficient was acquired and used as a reference. The same settings and transducer used in the clinical scan were used in the reference scan. Digital data of the beam-formed image were saved in Digital Imaging and Communications in Medicine (DICOM) format on a flash drive and converted to RF data on a personal computer using a Matlab® program supplied by Zonare. Attenuation estimates were obtained using an algorithm that was independently validated using tissue-mimicking ultrasonic phantoms.

Results RF data were acquired and analyzed to estimate attenuation of the human pregnant cervix. Regression analysis revealed that attenuation was: a predictor of the interval from ultrasound examination to delivery (β = 0.43, P = 0.01); not a predictor of gestational age at time of examination (β = −0.23, P = 0.15); and not a predictor of cervical length (β = 0.077, P = 0.65).

Conclusions Ultrasonic attenuation estimates have the potential to be an early and objective non-invasive method to detect interval between examination and delivery. We hypothesize that a larger sample size and a longitudinal study design will be needed to detect gestational age-associated changes in cervical attenuation. Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Preterm birth is a leading cause of infant morbidity and mortality worldwide. Assessment of uterine contractility and cervical change by digital examination have been considered to be a method to diagnose preterm labor. Since the introduction of tocolytics, preterm birth rates have not been reduced and the medications carry significant maternal and fetal risks. Evidence suggests that ripening of the cervix precedes uterine contractions and subsequent delivery of the fetus. Cervical ripening is a process characterized by collagen disorganization, decreased collagen concentration and increased water content. A ripened cervix, with loss of the mucus barrier, may be the initial mechanism leading to a cascade of events (increased inflammation, production of cytokines and enzymes, and loss of cervical mucus) that result in preterm birth. Thus, maintaining a closed unripe cervix with an intact cervical mucus barrier during pregnancy is critical for preventing preterm birth. Cervical changes that occur before preterm birth go undetected because women do not have symptoms (contractions) or signs (vaginal discharge). Measurement of early cervical changes (increased collagen disorganization and decreased collagen concentration) associated with remodeling of the extracellular matrix then becomes a medically significant milestone that may predict preterm labor and birth.

There is considerable interest in developing new imaging techniques to accurately predict cervical changes associated with full-term and preterm labor.

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Cervical shortening and dilation can be detected using clinical ultrasound systems. However, an objective non-invasive ultrasonic method to determine tissue property changes (increased collagen disorganization and water concentration) associated with cervical ripening does not exist. Estimates of ultrasonic attenuation (the loss of energy as an ultrasonic wave propagates through tissue)\(^{27}\) have been used to evaluate the structure and function of tissues in health and disease\(^{28–36}\). Attenuation has been observed to be related to tissue stiffness, collagen concentration and water concentration\(^{28–31,37}\). During pregnancy, as the collagen-rich cervix prepares for labor and birth, the cervix transforms from a rigid structure to a soft, extensible structure. It is thus hypothesized that by estimating changes in ultrasonic attenuation, these dynamic changes in tissue structure and function can be detected.

Our previous work in an animal model demonstrated that the decreased collagen concentration associated with cervical ripening clearly affected ultrasonic attenuation in cervical tissue\(^{18}\). We found that estimates of attenuation corresponded to gestational age changes in the rat cervix as ripening occurred\(^{18}\). In the rat cervix, attenuation was greater in the non-pregnant cervix and at an early gestational age, and decreased as pregnancy advanced towards full-term\(^{18}\). We attributed those changes in attenuation to changes in collagen disorganization\(^{13,18}\). Figure 1 presents atomic force microscopy images of a non-pregnant rat cervix, showing tightly packed and organized collagen bundles, and a day-21 pregnant rat cervix (the day on which rats typically deliver), showing highly disorganized collagen. Similar changes in collagen disorganization occur in human pregnancy as the cervix ripens\(^{38}\). We postulate that the development of methods to detect cervical attenuation in human pregnancy might be a useful means of estimating cervical tissue-property changes associated with cervical ripening.

Recently, a clinical ultrasound system (z.one; Zonare\(^{\text{TM}}\) Medical Systems, Inc., Mountain View, CA, USA) became available that allows access to radiofrequency (RF) image data of the human pregnant cervix similar to our previous animal studies\(^{18,39}\). Our previous studies in the animal model used ultrasound research data-acquisition systems (Panametrics, Waltham, MA, USA) and single-element high-frequency transducers (35 and 65 MHz)\(^{18,39}\). We wanted to extend our previous work, namely detecting cervical tissue changes in the animal model, to those changes occurring in human pregnancy by estimating ultrasonic attenuation of the human pregnant cervix with a clinical ultrasound system using a phased-array transducer. The purpose of this study was to develop a method to estimate ultrasonic attenuation in the human pregnant cervix using a clinical ultrasound system that provided access to RF image data for processing and analysis.

**METHODS**

Forty women underwent a cervical ultrasound examination once during pregnancy, using the z.one clinical ultrasound system. No longitudinal assessments of cervical attenuation were conducted on the women who participated in the study. The gestational age at the time of the cervical ultrasound examination ranged from 10 to 41 weeks. The inclusion criteria were: women with a live intrauterine pregnancy of between 10 and 42 weeks of gestation; and willingness to participate in the study. Women with a cerclage, who had undergone a loop electrosurgical excision procedure (LEEP) or a cone biopsy, or who had experienced rupture of membranes, vaginal bleeding or a fetal death, were excluded from the study.

All ultrasound data were acquired by one investigator (B.L.M.) with the same system presets for each examination. Two sagittal scans of the cervix, which included visualization of the internal and external os, were acquired for each woman using a 4–9-MHz (6.8-MHz center frequency) endovaginal transducer. Attenuation estimates and cervical length measurements were conducted for each of the two images on each woman’s cervix. Care was taken to obtain the scan in the same manner each time. Cervical length was measured, from the internal os to the external os, on the same image on which data would be analyzed for ultrasound attenuation. In addition, a scan of a tissue-mimicking phantom (University of Wisconsin, Madison, WI, USA), acquired with the same 4–9-MHz endovaginal transducer, was used to obtain the reference ultrasound signal for use when estimating cervical attenuation. The tissue-mimicking phantom was custom-made using 45- to 53-\(\mu\)m glass beads in agar and has been well characterized\(^{40}\) and used to obtain reference ultrasound data for research at the Bioacoustics Research Laboratory at the University of Illinois at Urbana-Champaign (Urbana, IL, USA). The attenuation coefficient of the tissue-mimicking phantom was

**Figure 1** Atomic force microscopy images of the cervix from (a) a non-pregnant rat, displaying highly organized, pack bundles of collagen and (b) a cervix on day 21 of pregnancy, displaying disorganized collagen with space between the fibrils. The bundles of collagen that are displayed in these images are \(\approx 0.1 \mu m\). Rats typically deliver on day 21. (Image by R. Bhargava, W. King, UIUC, and B. L. McFarlin, UIUC).
0.64 dB/cm-MHz and varied by as much as 0.15 dB/cm-MHz when evaluated among eight ultrasound research laboratories\(^4^0\). Figure 2 displays an ultrasound image of a tissue-mimicking phantom obtained with the 4–9-MHz endovaginal transducer used in this study. The mean attenuation of the phantom, measured using the endovaginal transducer, was 0.64 dB/cm-MHz (± 0.13 dB/cm-MHz). The settings of the ultrasound system were identical between the human scan and the reference phantom scan. The performance characteristics of the Zonare Medical Systems 4–9-MHz endovaginal transducer are summarized in Table 1.

Digital Imaging and Communications in Medicine (DICOM) data of the beam-formed image were saved on a flash drive and converted to RF data on a personal computer using a Matlab\(^\text{®}\) (Mathworks, Natick, MA, USA) program supplied by Zonare. Gestational age of the fetus was determined from the first-trimester ultrasound examination. Cervical length was measured at the time of the examination. Pregnancy characteristics and previous history of preterm birth were collected via patient interview. None of the women was subjected to a digital examination of the cervix. Gestational age at delivery and neonatal outcome data were acquired by telephone verbal report from the patient because most of the women delivered at various institutions throughout the states of Illinois and Indiana. All of the women were cognisant of infant birth weight, Apgar scores and date of delivery.

The attenuation estimates were obtained using an algorithm that was previously validated in tissue-mimicking ultrasound phantoms\(^4^1\). Briefly, backscattered RF waveforms were obtained from an unknown sample and from a reference phantom with a known attenuation coefficient, using the same transducer and system settings. Attenuation is defined as the loss of signal amplitude with depth as a function of frequency, and attenuation coefficient is the attenuation normalized to distance and/or frequency. The power spectra of both the reference phantom and the unknown sample are functions of frequency and propagation depth in the sample. The frequency dependence of the power spectra depends on the system transfer function, attenuation, scattering and diffraction of sound waves. The system transfer function and the diffraction term are approximated to be the same for both the reference sample and the phantom and can be eliminated by dividing the power spectra from the two samples and taking the logarithm. Determining the rate of change with depth into the tissue of this ratio for each frequency eliminates the scattering terms, assuming that the scattering properties do not vary with depth over the region of interest (ROI). Averaging the remaining coefficients with respect to frequency, and adding the attenuation coefficient of the reference phantom, then gives an estimate of the attenuation coefficient (dB/cm-MHz) in the unknown sample.

One investigator (B.L.M.) chose the ROIs for obtaining attenuation estimates. The ROIs were chosen based upon adequate size of ROI in homogeneous tissue (no cystic areas or amniotic fluid). Because of the tissue architecture, the same ROI could not be sampled in each image. Therefore, we used one attenuation estimate value for each patient. The investigators processing the RF data (T.A.B. and Y.L.) were blinded to gestational age and clinical information about the patients. In order to obtain an error of less than 10%, the ROI must be at least 40 wavelengths (1 cm in length for a 6-MHz transducer) and contain at least 30 beam lines. Institutional Review Board approval of the study was obtained at Rush University and at the University of Illinois at Chicago.

The purpose of this study was to develop methodology to estimate attenuation using a clinical ultrasound system. Hence, the study was not powered to detect differences in attenuation and cervical length as a function of gestational age at the time of examination. However, descriptive statistics and one-way analysis of variance (ANOVA) (two-tailed tests) were calculated for gestational age at the time of examination (SPSS 16.0; SPSS, Chicago, IL, USA). Scatterplots with linear regression lines and 95% CI were calculated to display the relationship between attenuation and interval to delivery, cervical length and gestational age at the time of examination. An alpha level of 0.05 was used for all statistical tests.

![Figure 2 Attenuation map for a B-mode image obtained from the tissue-mimicking phantom (the phantom has a known attenuation coefficient of 0.64 dB/cm-MHz) using a 4–9-MHz endovaginal transducer (z.one; Zonare Medical Systems, Inc.). Each pixel represents a mean attenuation coefficient value for a region of interest. The mean (± SD) attenuation coefficient measured for the phantom was 0.64 (± 0.13) dB/cm-MHz.](image)

**Table 1** Transducer performance characteristics as reported by Zonare Medical Systems, Inc

<table>
<thead>
<tr>
<th>Transducer property</th>
<th>Endovaginal 4–9 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of elements</td>
<td>128</td>
</tr>
<tr>
<td>Center frequency (6 dB)</td>
<td>6.4 MHz (± 0.2)</td>
</tr>
<tr>
<td>Bandwidth</td>
<td>≥ 65%</td>
</tr>
<tr>
<td>Focal length</td>
<td>33 mm (± 10)</td>
</tr>
<tr>
<td>Pulse width (6 dB)</td>
<td>≤ 0.24 μs</td>
</tr>
</tbody>
</table>

RESULTS

Forty women participated in the study. Seven women delivered at < 37 weeks of gestation. Table 2 displays the characteristics of women in the study. Regression analysis revealed that attenuation was: (1) a predictor of the interval from ultrasound examination to delivery ($\beta = 0.43, P = 0.011$); (2) not a predictor of gestational age at time of examination ($\beta = -0.23, P = 0.15$); and (3) not a predictor of cervical length ($\beta = 0.077, P = 0.65$). Figure 3 displays scatterplots of cervical-attenuation estimates as a function of interval from ultrasound examination to delivery, gestational age at time of examination and cervical length, for the women in the study. Table 3 displays the characteristics of the women in the study who delivered preterm. Figure 2 displays the attenuation coefficient of the tissue-mimicking phantom (mean = 0.64 dB/cm-MHz, SD = 0.13 dB/cm-MHz). Similar attenuation estimate results were reported by other investigators who used this phantom.

Table 4 summarizes the characteristics of women who had a cervical length of < 2.5 cm at the time of the ultrasound examination. Patient no. 35 had a very short cervix, a moderate attenuation value and did not deliver for 11 more weeks. She was monitored and did not receive tocolytics as she was not contracting. Patient no. 41 was noted to have a cervical length of 2.26 cm at 18 weeks, a moderately high attenuation value and did not deliver for 19 more weeks. Figure 4 displays images of the cervices of two women who participated in the study – one at 14 weeks of gestation and one at 38 weeks of gestation. Ultrasound B-mode images of the cervices (Figures 4a and c) of the two women were created along with corresponding superimposed maps of the attenuation estimates for the cervices (Figures 4b and d). Each pixel in the superimposed image represents a mean attenuation value for the intended ROI of the cervix. We wanted to evaluate both the internal and external portions of the cervix, but this was not always possible because of inhomogeneities and architecture variances of the cervical tissue. In the two cases presented in Figure 4, mean attenuation was greater in the 14-week cervix than in the 38-week cervix, although there was little change in cervical length measurements (3.1 cm for the 14-week cervix and 3.5 cm for the 38-week cervix).

Like the cervical attenuation estimates in our previous animal studies, considerable between-subject attenuation variability was noted (Figure 3a). Unlike attenuation estimates in the previous animal cervical studies, however, the attenuation variability across the cervix for a specific patient was reduced compared with that in rats as a result of the larger ROIs sampled in the larger human cervix.

Table 2 Characteristics of the 40 study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>All women (n = 40)</th>
<th>Women delivering at ≥ 37 weeks (n = 33)</th>
<th>Women delivering at &lt; 37 weeks (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>28 ± 6.7 (19–42)</td>
<td>28 ± 6.8 (19–42)</td>
<td>28 ± 8.4 (19–38)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1–5)</td>
<td>2 (1–5)</td>
<td>2 (1–4)</td>
</tr>
<tr>
<td>History of PTD</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>History of PTD</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>GA at ultrasound (weeks)</td>
<td>22.5 ± 8.6 (10–40)</td>
<td>22.8 ± 8.9 (10–40)</td>
<td>23.4 ± 8.1 (12–35)</td>
</tr>
<tr>
<td>Attenuation (dB/cm-MHz)</td>
<td>1.19 ± 0.36 (0.31–1.9)</td>
<td>1.24 ± 0.31 (0.56–1.9)</td>
<td>1.06 ± 0.48 (0.31–1.62)</td>
</tr>
<tr>
<td>Interval from ultrasound to delivery (weeks)</td>
<td>14.6 ± 8.7 (0–29)</td>
<td>15.8 ± 8.2 (0–29)</td>
<td>9.4 ± 9.3 (1–24)</td>
</tr>
<tr>
<td>Cervical length (cm)</td>
<td>3.7 ± 1.32 (0.56–6.1)</td>
<td>3.8 ± 1.33 (0.56–6.1)</td>
<td>2.9 ± 1.53 (0.74–5.1)</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>37.6 ± 3.1 (27–41)</td>
<td>38.8 ± 1.1 (37–41)</td>
<td>32.8 ± 3.8 (27–36)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2970 ± 7.95 (843–4223)</td>
<td>3224 ± 475 (2300–4223)</td>
<td>1891 ± 849 (843–3288)</td>
</tr>
</tbody>
</table>

Data are expressed as n, mean ± SD (range) or median (range). GA, gestational age; PTD, preterm delivery (< 37 weeks); PTL, preterm labor (< 37 weeks).

Table 3 Characteristics of the seven study participants who delivered preterm

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gravidity (FPAL)</th>
<th>History of preterm birth</th>
<th>GA at ultrasound (weeks)</th>
<th>GA at delivery (weeks)</th>
<th>Cervical length (cm)</th>
<th>Mean attenuation (dB/cm-MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
<td>No</td>
<td>35</td>
<td>36</td>
<td>2.63</td>
<td>0.55</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>No</td>
<td>32</td>
<td>34</td>
<td>3.06</td>
<td>1.62</td>
</tr>
<tr>
<td>17</td>
<td>2</td>
<td>Yes</td>
<td>12</td>
<td>36</td>
<td>4.89</td>
<td>1.23</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>No</td>
<td>24</td>
<td>27</td>
<td>2.97</td>
<td>0.316</td>
</tr>
<tr>
<td>32</td>
<td>2</td>
<td>No</td>
<td>26</td>
<td>28</td>
<td>3.10</td>
<td>1.49</td>
</tr>
<tr>
<td>33</td>
<td>1</td>
<td>No</td>
<td>13</td>
<td>36</td>
<td>5.10</td>
<td>1.21</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>No</td>
<td>23</td>
<td>34</td>
<td>1.24</td>
<td>1.06</td>
</tr>
</tbody>
</table>

FPAL, full-term, preterm, abortion, living; GA, gestational age.

between benign and malignant tissue in the prostate and assessing the mineral content of bone. Attenuation of tissue has been observed to decrease significantly as water concentration increases and density decreases. With the dynamic changes in cervix microstructure (increased collagen disorganization and decreased collagen concentration) and water concentration during the ripening process, it is reasonable to consider using attenuation to detect changes in the cervix that are associated with cervical ripening. Our own research in the rat cervix observed significant decreases in attenuation as pregnancy progressed towards full-term, as well as significant correlations of attenuation with cervix tissue water concentration. Significant between-subject variability in attenuation was observed. Our previous rat-cervix attenuation estimates also showed marked between-subject variability, even though the rat pregnancy gestational age is known to within 12 h of conception, suggesting biologic variation in cervical tissue starting points. These findings suggest that future longitudinal repeated-measure designs will be needed to detect cervical change leading to labor, as each patient may have her own cervix attenuation starting point. It is possible that the patterns of cervical change will be different in women delivering at full-term or at preterm. It is promising that in this small sample size, attenuation estimates were associated with interval to delivery. There may be a critical level of attenuation that corresponds to signaling of impending labor. Note that the cervical length in the Figure 4 images was not markedly different, but the attenuation values and gestational age were very different. The lack of relationship between cervical-attenuation estimates and cervical length suggests that the process of microstructural tissue property changes and cervical length may be independent of, or at least not concurrent with, attenuation.

Limitations

The goal of this research was to construct attenuation maps of the entire cervix, or at least be consistent in sampling the internal and external portions of the cervix. The attenuation algorithm assumes homogeneous tissue. We selected cervical ROIs that appeared to be homogeneous tissue. We selected cervical ROIs that appeared to be homogeneous tissue, without any cystic areas. Because of the cervical tissue architecture, the same ROIs could not be uniformly sampled in each individual case. Therefore, we used one of the two cervical images we collected in each case to produce the results presented in this study. This undersampling of the cervix is a limitation of our approach given the natural inhomogeneity of the cervix. This also may have increased the between-subject variability because the same regions of the cervix were not compared in every case. In future studies, our goal will be to develop methods to obtain attenuation estimates throughout the entire cervix.

A further limitation of the study is that the attenuation estimation algorithm assumes that the ROI is...
Table 4 Characteristics of the five study participants with a cervical length of < 2.5 cm at the time of examination

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gravidity</th>
<th>Parity (FPAL)</th>
<th>History of preterm birth</th>
<th>GA at ultrasound (weeks)</th>
<th>GA at delivery (weeks)</th>
<th>Cervical length (cm)</th>
<th>Mean attenuation (dB/cm-MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>2</td>
<td>0100</td>
<td>Yes</td>
<td>29</td>
<td>40</td>
<td>0.56</td>
<td>1.01</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>0000</td>
<td>No</td>
<td>38</td>
<td>38</td>
<td>0.94</td>
<td>0.65</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>2002</td>
<td>No</td>
<td>23</td>
<td>34</td>
<td>1.24</td>
<td>1.06</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1010</td>
<td>No</td>
<td>39</td>
<td>40</td>
<td>1.62</td>
<td>1.04</td>
</tr>
<tr>
<td>41</td>
<td>2</td>
<td>0101</td>
<td>Yes</td>
<td>18</td>
<td>37</td>
<td>2.26</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Results are ordered according to increasing cervical length. FPAL, fullterm, preterm, abortion, living; GA, gestational age.

Figure 4 Ultrasound B-mode images (a,c) and B-mode images superimposed with maps of the mean attenuation estimates (b,d) of two women in the study, one at 14 weeks of gestation, with cervical length 31 mm (a,b) and the other at 38 weeks of gestation, with cervical length 35 mm (c,d). The colors of the regions of interest represent a mean attenuation (dB/cm-MHz) consistent with the corresponding scale bar.

homogeneous (i.e. has the same attenuation and scattering properties). Therefore inhomogeneities within the ROI could result in errors in the attenuation estimate. The best way to reduce these errors is to increase the ROI size. Because the minimal ROI size needed for accurate estimates is a function of wavelength, increasing the ultrasound frequency (reducing the wavelength) is a relatively simple approach for reducing the sensitivity to inhomogeneities. The smaller ROIs obtained at higher ultrasound frequencies could also enhance our ability to obtain attenuation estimates at late gestational ages when the cervix has a shorter length as a result of effacement42.

The small sample size of this study limits the generalizability of our findings. We are encouraged by our preliminary findings that it may be possible to detect early cervical tissue property changes in human pregnancy that lead to labor. In the future, we plan to conduct studies with longitudinal repeated-measures designs to assess cervical

attenuation throughout pregnancy as a measure of the cervix preparing for labor.

Conclusions

It is clear that we still have much to learn about the mechanisms and processes of cervical ripening. New imaging technologies are necessary to fully understand and detect such changes during pregnancy. Significant technological advances in computing and instrumentation have made it possible to improve the detection of tissue-property changes associated with cervical ripening. We postulate that it will be possible to add attenuation assessment processing software to current clinical ultrasound systems with RF data capabilities. Attenuation estimates have the potential to be an early and objective non-invasive method of detecting changes in cervical tissue microstructure that are consistent with cervical ripening.

ACKNOWLEDGMENTS

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