Assessment of the in vivo biomechanical properties of the human uterine cervix in pregnancy using the aspiration test
A feasibility study
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1. Introduction

The course and outcome of pregnancy are closely related to the biochemical and biomechanical functions of the cervix. Understanding the physiologic remodeling process of the cervix during gestation is crucial for the development of methods which control the cervical function and accurately diagnose preterm labor and cervical insufficiency (CI). Recently structural changes of the cervical stroma were shown during pregnancy using magnetic resonance imaging [1]. Preterm cervical ripening associated with cervical insufficiency may be associated with preterm birth, while a long, unripe cervix at term is treated with induction of labor, that, when unsuccessful is the reason for cesarean section. Rechberger et al. [2] identified a relationship between concentrations of collagen and hyaluronic acid and cervical dilatation time during labor in primiparas. It was shown that a long cervix at mid-trimester increases the risk of cesarean section at term [3]. In clinical practice (i) vaginal ultrasound is applied to estimate cervical properties of the cervix in pregnancy with the aim to describe the physiological biomechanical changes throughout gestation in order to eventually detect pregnant women at risk for cervical insufficiency (CI). Methods: We show the first clinical application of an aspiration device to assess the in vivo biomechanical properties of the cervix in pregnancy with the aim to detect pregnant women at risk for cervical insufficiency (CI). Results: Out of 15 aspiration measurements, 12 produced valid results. The stiffness values were in the range between 0.013 and 0.068 bar/mm. The results showed a good reproducibility of the aspiration test. In our previous test series on non-pregnant cervices our repetitive measurements showed a standard deviation of >20% compared to 9.10% to our data on pregnant cervices. Stiffness values are decreasing with gestational age which indicates a progressive softening of cervical tissue towards the end of pregnancy. Three pregnant women had two subsequent measurements within a time interval of four weeks. Decreasing stiffness values in the range of 20% were recorded.

Discussion: This preliminary study on the clinical practicability of aspiration tests showed promising results in terms of reproducibility (reliability) and clinical use (feasibility). Ongoing studies will provide further insights on its usefulness in clinical practice and in the detection of substantial changes of the cervix in pregnancy indicative for threatened preterm birth or cervical insufficiency.

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2. Pathologic conditions related to the cervix

2.1. Cervical insufficiency (CI)

Several studies have documented a disturbed biochemical and biomechanical integrity in women with CI, even when they are not pregnant [6,7]. For example, a biopsy study on non-pregnant cervices performed in women with a history of CI clearly demonstrated a significant lower hydroxyproline concentration and increased hydroxyproline extractability [6]. Insufflation tests inserting a balloon into the cervical canal revealed pressure–volume characteristics indicating a significant lower resistance in women with previous spontaneous midterm pregnancy losses, preterm birth or clinically diagnosed cervical insufficiency [7]. Cervical biopsy specimens taken from patients with cervical insufficiency contained normal collagen concentrations, but relatively high collagen extractabilities and collagenolytic activities during the second trimester, whereas biomechanical testing revealed low strength and high extensibility [2]. Elastin, another component of the extracellular matrix, was found at a lower content in the incompetent cervix which suggests that cervical elastin is also responsible for keeping the cervix closed and undilated throughout gestation [8].

Diagnosis of CI is based on the measurement of a shortened cervix by vaginal ultrasound examination in the absence of uterine contractions. Recently it was shown that the cervix demonstrated a dynamic response on vaginal ultrasound in relation to varying intrauterine pressure, which makes examination in different positions of the pregnant woman reasonable [9].

2.2. Preterm labor

Differentiation between true and false labor is sometimes difficult especially in the presence of a normal cervix. Pregnant women experience contractions related to uterine growth without cervical changes and without risk of preterm delivery. Screening tests such as digital and ultrasound evaluations of the cervix and assays for fetal fibronectin in cervicovaginal secretions had a low sensitivity and positive predictive value for preterm labor [10]. It was shown that in women with threatened labor ultrasonographic measurements of the cervical length were useful in the distinction between true and false labor and in the prediction of early preterm delivery [11]. Women with a cervical length of <25 mm and a contraction frequency ≥4 per hour in the second trimester had an increased risk of spontaneous preterm birth [12]. Uterine electromyography was a useful tool to record preterm and term labor and showed a specific pattern of activity [13], but prospective studies are awaited to objectively judge its power in clinical practice. The use of the aspiration test might provide useful information to discriminate between true and false labor.

3. Subjects and methods

3.1. Aspiration test

In a multidisciplinary approach we have recently introduced an aspiration test to assess the biomechanical function of the uterine cervix [14,15]. The technical equipment of the aspiration device and the working mode were described recently [14,15]. An image of the instrument and a drawing with the detail of the distal end of the aspiration tube in contact with the tissue is shown in Fig. 1. For the use on pregnant women, measurements were taken by using a miniaturized version of the aspiration device [16] with an external diameter of 15 mm and an aspiration hole of 8 mm in diameter.

3.2. Clinical study at the University Hospital Zurich

This is an ongoing clinical study at the Clinic of Obstetrics, Dept. OB/GYN, University Hospital of Zurich which aims at quantifying the mechanical response of normal cervical tissue at different gestational ages. The study was approved by the ethical commission of the Canton of Zurich. Informed consent was obtained from each pregnant woman who participated in the study. Measurements were performed by a gynecologist during standard gynecological examinations.

3.3. Testing protocol

A preliminary step was performed with a slow ramp loading for determining the maximum suction pressure to be safely applied on the cervical tissue. The maximum value of negative relative pressure was determined so that the deformation of the sucked tissue did not exceed a maximum displacement of 4 mm. Subsequently a loading cycle was performed with a rapid ramp...
from atmospheric to a maximum negative pressure, held at this pressure level for about 8 s, and then released to atmospheric pressure. The total duration of the cycle was 25 s. Insertion and placement of the aspiration device was guided by application of a speculum. Care was taken to place the device on the anterior lip of the cervix, at 12 o’clock position, not overlying columnar epithelium (to minimize the risk of bleeding). Twenty measurement sessions took place so far and no subject reported pain; no irritations, bleeding or trauma were observed at visual inspection after the measurements. In a few cases the patient reported discomfort related to the total duration of the examination. In fact, despite the short duration of the aspiration measurements (few minutes) the time period was about twice as long as standard gynecological examinations. For this reason the measurement protocol (originally planned with 3–4 cycle repetitions and one multi-cycle experiment) was eventually minimized, with one, maximum two aspiration cycles applied in each session.

Measurements were taken between 21 and 36 weeks of gestation. In 5 out of 20 cases no aspiration measurements were performed due to technical problems (for example, failing calibration of the pressure sensor) or due to non suitable placement of the aspiration device on the tissue. The latter were caused by either excessive compressive force applied onto the cervix (too large pre-deformation of the tissue) or too weak contact, leading to air leakage.

3.4. Stiffness parameter

The deformation profiles obtained from the aspiration experiments were processed and the displacement history of the highest point of the profile was extracted. Fig. 2 shows two examples of measurement data, with the applied pressure history and the corresponding measured displacement at the highest point of the profile. The two diagrams correspond to data obtained from repeated aspiration experiments performed in the same session. The points A, B and C and the distance $d$ are indicated in both diagrams.

Point A: displacement at initial contact, before the pressure decreases.
Point B: displacement where minimum pressure is applied.
Point C: maximum displacement, before pressure release.
$d$: displacement at C minus displacement at A.

For data analysis we calculated the stiffness parameter $\eta$ according to

$$\eta = \frac{p_{\text{min}}}{d}$$

where $p_{\text{min}}$ is the smallest pressure applied.

4. Results and interpretation

Out of 15 aspiration measurements, 12 produced valid results. In three cases the image quality was not sufficient to extract the tissue profile and thus quantitatively assess tissue deformation. For three pregnant women, measurements were taken in two subsequent sessions, with the second measurement four weeks after the first one. All measured values of the stiffness parameter $\eta$ (in bar/mm) available so far as a function of the corresponding gestational age (in weeks) are reported in Fig. 3. The stiffness values were in the range between 0.013 and 0.068 bar/mm. In three cases two aspiration cycles were applied. The average of the measurements is given along with vertical bars indicating the position of the corresponding measured values (Fig. 3). A trend line is also reported in the diagram of Fig. 3. The line corresponds to a linear fit of all data points. Regarding the repeatability of the test results we compared our previous test series on non-pregnant cervices [14] where our repetitive measurements showed a standard deviation of >20% compared to our recent data, i.e. <±10%. Decreasing values of stiffness parameter with gestational age correspond to the expectation showing a progressive softening of cervical tissue towards the end of pregnancy. This indication is confirmed by the three cases with sequential measurements at four weeks time interval, as shown in Fig. 4. A consistent decrease in the range of 20% with respect to the first measured stiffness value is observed in all three subjects.
5. Discussion

During the last decade different approaches have been pursued to objectively verify cervical changes in pregnancy posing a woman at increased risk for preterm birth. For example, House and Socrate [17] proposed the cervix as a biomechanical structure that provides strength in the presence of active and passive loads. Various diagnostic tools based on a variety of physical phenomena have been tested for their use in clinical practice. A cervical resistance monitor was introduced to define the cervical resistance index while the cervix is dilated to 8 mm [18]. This instrument was successfully used to evaluate the cervix in women with a history of spontaneous mid-trimester losses. The drawback of this method is that its use in pregnancy is limited since the examination required general anesthesia and the application of a device into the cervical canal is rather invasive with the risk to induce rupture of membranes or inflammation. Other encouraging methods tested were electrical impedance measurements [19,20], and light induced fluorescence [13,21], which are both indirect estimates of the cervical ripening process, and hence it is not completely clear how conclusive these data are to characterize the biomechanical properties of the cervix. McFarlin et al. [22] tested a quantitative ultrasound technique on rat cervices that was useful in the detection of the ripening process. Another technique was developed where a cervicotonometer was applied to the cervix. An elevated distensibility index was a good predictor for preterm birth. For example, House and Socrate [17] proposed the cervix as a biomechanical structure that provides strength in the presence of active and passive loads. Various diagnostic tools based on a variety of physical phenomena have been tested for their use in clinical practice. A cervical resistance monitor was introduced to define the cervical resistance index while the cervix is dilated to 8 mm [18]. This instrument was successfully used to evaluate the cervix in women with a history of spontaneous mid-trimester losses. The drawback of this method is that its use in pregnancy is limited since the examination required general anesthesia and the application of a device into the cervical canal is rather invasive with the risk to induce rupture of membranes or inflammation. Other encouraging methods tested were electrical impedance measurements [19,20], and light induced fluorescence [13,21], which are both indirect estimates of the cervical ripening process, and hence it is not completely clear how conclusive these data are to characterize the biomechanical properties of the cervix. McFarlin et al. [22] tested a quantitative ultrasound technique on rat cervices that was useful in the detection of the ripening process. Another technique was developed where a cervicotonometer was applied to the cervix. An elevated distensibility index was a good predictor for preterm delivery [23]. This method is interesting in comparison to our approach since it represents an inverse technology. The cervicotonometer estimates the mechanical properties of a tissue by compression whereas the aspiration test by suction. It is also worth noting that Cabrol [24] reports a distensibility index that is based on the values of the force used to open the cervix and the inner diameter of the cervix obtained with each force used.

In the present study we introduced an aspiration device, which, as a diagnostic tool, might directly assess the biomechanical properties of the cervix during pregnancy. In this study we found that the device was well tolerated and no immediate complications were observed. This, in fact, represents the pre-requisite for future clinical investigations to further evaluate the clinical relevance of this technique for diagnosis.

The number of women tested in this preliminary study is yet too low in order to draw a conclusion on the significance of our data and their quantitative relation with the evolution of the mechanical response of cervical tissue during gestation. However, as expected, the measured response showed a trend towards decreasing stiffness during the course of pregnancy. Based on this encouraging initial phase of our study data collection will continue and will hopefully provide meaningful information on the stiffness parameters during the course of gestation. Knowledge on the physiologic biomechanical behavior will facilitate the recognition of early pathologic conditions. Stiffness data from the second trimester and from term are of special interest to (i) differentiate between true and false labor, to (ii) estimate the risk of preterm birth due to cervical insufficiency and to (iii) estimate the success for induction of labor and the risk of cesarean section due to cervical unripeness.

The stiffness values identified in pregnant women are in the range between 0.013 and 0.068 bar/mm. The average stiffness parameter for cervices from post-menopausal women was approximately 0.14 bar/mm [14,15]. The mechanical response of pregnant cervices observed in this study is considerably softer compared with post-menopausal; a result which may be explained by the physiologic remodeling process of the cervical extracellular matrix. This result is in concordance to the biomechanical testing of cervices derived from pregnant and non-pregnant hysterectomy specimens where non-pregnant tissue was significantly stiffer than pregnant tissue in both tension and compression [25].

However, a quantitative comparison of the present values with the data from our previous study should not be carried out. In fact, (i) the present data were obtained with an 8 mm diameter aspiration device compared with 10 mm diameter of the previous device, and (ii) a different value of the maximum negative pressure was applied in each experiment of the current study, whereas a constant value of 220 mbar was applied in our former study. Data of the present study also indicate a better repeatability of the results which might be related to the smaller design of the device which is better to handle.

At the current stage of our trial we are aware of potential pitfalls. Since the aspiration device is placed at squamous epithelium which shows a thickness between 0.5 and 1 mm and our measurements reach a tissue displacement of the same range it might be questioned whether this test provides information of the cervical stroma. The squamous epithelium and the cervical stroma are tightly connected to each other forming a composite. Hence, by applying a negative pressure, this composite will respond with a kind of “smeared” stiffness/compliance, very much driven by the mechanical properties of the voluminous stroma. Another issue is the variable force with which the device is applied to the cervix that would influence the results of the stiffness parameter. Therefore, the device was carefully applied without pre-compressing the tissue. For the future it would be beneficial to use a kind of sensor in order to detect and standardize the (small) initial pressure.

We conclude that the aspiration technique allows the detection of changes in the cervical stiffness which is assumed to reflect the ripening process. Our ongoing study will provide further information on its usefulness in clinical practice and in the detection of substantial changes of the cervix in pregnancy indicative for threatened preterm birth or cervical insufficiency. Future studies will also address a correlation of our proposed aspiration technology with ultrasound parameters (cervical length and funneling) and biochemical markers for threatened preterm birth (e.g., fibronectin).

Conflict of interest

We have nothing to declare in any of these categories.

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References


