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ORIGINAL RESEARCH

Investigation of Uterine Contractility and Myometrial Stiffness in Pelvic Organ Prolapse

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Purpose: Pelvic organ prolapse (POP) is a common condition affecting women's quality of life. The pathophysiology of POP is complex and not yet fully understood. This study aimed to investigate uterine contractility and myometrial stiffness in patients with POP.

Patient Methods: The study included 27 POP patients and 27 non-POP patients scheduled for hysterectomy. Preoperatively, transabdominal shear wave elastography (SWE) examinations were performed. During hysterectomy, myometrial tissues were collected and suspended in isolated organ baths. Peak-to-peak (p-p) contractions, the area under the curve (AUC), and frequency changes before and after oxytocin application were normalized as percentage changes between the two groups.

Results: No significant differences were observed in SWE measurements between the groups. When uterine contraction measurements were compared after oxytocin administration, frequency, p-p, and AUC values were statistically significantly reduced in the POP group compared to the non-POP group ($p < 0.05$).

Conclusion: An association was observed between decreased uterine contractility and POP. The results obtained in the present study suggest that decreased uterine contractility may be a risk factor for the development of POP.

Keywords: pelvic organ prolapse, isolated organ bath, shear wave elastography, uterine contractility

Introduction

Pelvic organ prolapse (POP) is defined as the herniation of the anterior and/or posterior vaginal wall, uterus, or vaginal apex into the vagina. However, descent may also be observed in one or more structures.[1] Although POP is more common in older women, it can affect women of all ages. The incidence of POP increases with age and can reach up to 5% in women aged 60–69 years.[2] At the same time, nearly 50% of women show some degree of prolapse on physical examination, and only 3% report POP symptoms.[2,3] Some studies suggest that prolapse progresses until menopause, after which the rates of progression and regression become lower.[2,4,5]

Pregnancy is the most frequently associated factor with POP; however, various factors can contribute to its etiology. Pelvic support is maintained predominantly by the levator ani muscles and their connective tissue attachments to the pelvic sidewalls of the vagina. Due to the effect of pelvic support, the vagina lies horizontally on the levator ani muscles. The levator ani muscles become more vertical position if the muscle damaged and the vaginal hiatus expands. Biomechanical modeling has shown that, the levator ani muscles are stretched beyond 200% of their tension threshold, during the second stage of labor increasing the risk of injury.[6]

To better understand POP, it is essential to comprehend pelvic floor dysfunction (PFD) and anatomy. PFD encompasses a broad spectrum of symptoms and anatomical changes associated with abnormal functioning of the pelvic floor muscles. Impaired function may result from increased activity (hypertonicity), decreased activity (hypotonicity), or improper coordination of the pelvic floor muscles. Changes in pelvic organ support are considered within the scope of PFD and are commonly referred to as POP. The pelvic floor is a complex structure composed of numerous muscles

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connected by ligaments, forming a dome-shaped diaphragm across the bony pelvic outlet. Conditions such as hypertonicity, hypotonicity, and loss of pelvic support are widely recognized as contributing factors.[7,8]

Concerns regarding the association between childbirth and PFD apply to women who have undergone either cesarean or vaginal delivery. Pelvic floor laxity may be related to inherited or acquired collagen abnormalities associated with pregnancy.[9] It remains unclear whether these changes are causes or effects of POP, as POP can also develop in nulliparous women without any apparent risk factors.[10] This is attributed to genetic factors and changes in the synthesis and degradation of different collagen and elastin types, linking POP development to deficiencies in the strength and elasticity of the suspensory and supportive tissues of the vagina and pelvic organs.[11,12]

In POP patients, complications such as cervical insufficiency, preterm birth, postpartum hemorrhage, uterine atony, and uterine inversion occur at higher rates compared to non-POP patients. This raises questions about whether the reduced elasticity observed in POP is also present in myometrial tissue. The underlying cause of POP-associated maternal complications is thought to be the loosening of connective tissue in the uterus and cervix, along with alterations in collagen content, type, and organization.[13]

The isolated organ bath system has been widely used in studies investigating myometrial physiology, pathology, and pharmacology. In this ex vivo technique, myometrial strips are suspended, and tension during contraction is measured. Parameters such as contraction frequency, speed (via peak-to-peak (p-p) measurements), duration, and force integral (area under the curve [AUC]) can be assessed.[14]

Sonoelastography (SE) is an ultrasound-based technique that evaluates tissue elasticity. By precisely measuring elasticity, SE provides valuable insights into tissue stiffness. In recent years, it has become a reliable method for diagnosing and monitoring disease progression, particularly in characterizing liver tissue and assessing liver-related conditions such as fibrosis and cirrhosis.[15,16] In gynecological practice, SE has been employed to evaluate the elasticity of the levator ani muscle and vaginal tissue, which are supporting components of the integral theory in POP patients. Studies have shown higher vaginal tissue and levator ani muscle elasticity in POP patients compared to non-POP patients when measured by SE.[17,18]

In this pilot study, we aimed to compare uterine contractility and myometrial tissue elasticity in patients with and without POP using SE and isolated organ bath methods.

Materials and Methods

This prospective pilot study was conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine, Fırat University, following approval from the Ethics Committee of Fırat University (number:2022/14-35). Written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki.

Sample Size

Based on myometrial elastography, minimum sample size was calculated as 27 individuals in each group to achieve 80% power with a significance level of 0.05 and an effect size (d) of 0.78.

Study Design

According to the diagnostic criteria of the POP quantification system, 27 women with uterine prolapse scheduled for surgery and 27 women without prolapse scheduled for hysterectomy due to benign reasons (benign ovarian tumour, postmenopausal bleeding, endometrial hyperplasia, cervical intraepitelial neoplasia and endometrial polyp) were included in the study. Exclusion criteria included the presence of adenomyosis or leiomyoma affecting uterine stiffness and contraction, a history of myomectomy or uterine surgery, abdominal fat thickness greater than 6 cm (which could interfere with transabdominal elastography measurements), and hormone use within the past 3 months. Before surgery, all patients underwent B-scan and transabdominal shear wave elastography (SWE) ultrasound examinations. During hysterectomy, full-thickness myometrial tissue (2 cm in length, 1 cm in width) was excised from the anterior corpus of the uterus and placed in a petri dish containing Krebs–Henseleit solution.

SWE Examination

The SWE examination was performed by a radiologist who is experienced in SWE and gynecological ultrasonography. Prior to surgery, all patients underwent evaluation at the Department of Radiology, Fırat University Hospital, using an ultrasound equipment with SWE software (GE Logic S8 xdCLEAR 2.0, Korea). The examination was conducted in the supine position after 5 minutes of rest. Using a 9–12 MHz convex probe, B-mode ultrasonography was performed, followed by SWE. Gray-scale and elastographic images were digitally recorded during the sonographic assessment. Since SWE is a dynamic sonoelastographic technique, no manual compression was applied. Patients were requested to not to breath during the examination to stabilize the image. The imaging field of view (FOV) was fixed on the myometrium of corpus anterior. Within the FOV, the region of interest (ROI) cursor was placed on the stiffest portion and ROI was established based on the stiff area. SW velocity measurements were taken within the ROI. After confirming that there were no significant differences between measurements performed using a single ROI and at least three ROIs, most lesion measurements were conducted with a single ROI. This process was repeated 10 times, and the median values were included in the study (Figure 1). The SW velocity of the lesions was converted to Emean in kilopascals (kPa) and recorded.[19]

Isolated Organ Bath

Organ Bath Contraction Experiments

Myometrial tissue samples measuring 3×8×12 mm were obtained for use in the isolated organ bath. These tissue sections were suspended under 2000 grams of tension in 5 mL isolated organ baths containing an electrolyte composition (KHS) with the following components (mM: 4.7 KCl, 1.2 $MgSO_4$, 118 NaCl, 1.18 KH_2PO_4, 2.4 $CaCl_2$, 15.8 $NaHCO_3$, 11.5 Glucose, 0.016 Ethylenediaminetetraacetate). The temperature of the organ bath was maintained at 37°C. The Krebs solution was continuously supplied with 95% O_2 and 5% CO_2. Every 30 minutes, the system was washed with Krebs solution to acclimatize the tissue to the in vitro environment. After a 120-minute regulation period, 10 µL of oxytocin was administered, and the effects of the drug were observed for 30 minutes. The system was then washed with Krebs solution, and 80 mM KCl was applied to confirm tissue viability before the experiment was terminated. Contraction forces were recorded isometrically using a transducer connected to an amplifier and data collection system. The p-p contractions, AUC, and frequency values of tissue contractions before and after oxytocin administration were measured.[20]

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0.2 and IBM SPSS Statistics version 27 software packages. The data were normalized as percentage changes, and the normality of distribution was confirmed with the Shapiro–Wilk test. Independent samples *t*-tests were used to compare the data between the non-POP and POP groups. Pearson chi-square analysis was used to compare categorical variables. Data were presented as mean±standard deviation for quantitative variables and frequency and percentage for categorical variables. A p-value of <0.05 was considered statistically significant for all analyses.

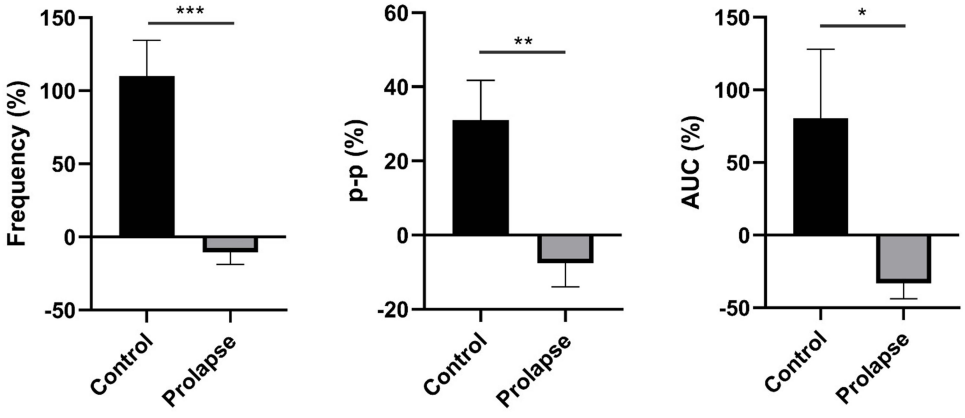

Figure 1 Data on uterine contractions after 10 µL oxytocin administration (Data are presented as mean ± ss, *p<0.05, **p<0.01, ***p<0.001).

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Table 1 Main Clinical Characteristics of the POP and Non-POP Groups

	POP (n:27)	Non-POP (n:27)	p Values
Age	59.19±10.01	55.11±10.63	0.153
Menopausal status			
Premenopausal	6 (22.2%)	8 (29.6%)	0.757
Postmenopausal	21 (77.8%)	19 (70.4%)	
POP-Q Stage	11 (40.7%)	-	
2			
3	4 (14.8%)	-	
4	12 (44.4%)	-	
Uterine size (mm)			
Length	68.22±16.54	73±18.93	0.328
Depth	42.63±13.42	47.15±16.11	0.268
Parity	4.22±2.15	4.19±2.11	0.949
Gravida	5.15±2.61	4.74±2.41	0.554
Elastography (kPa)	8.52 ±3.20	10.09±4.26	0.133

Notes: Data presented as mean±sd.

Results

A total of 54 cases, aged between 43 and 82 years, were included in the study. No significant differences were found between the two groups in terms of age, menopausal status, gravida, parity, and uterine size (Table 1).

When transabdominal SWE measurements were compared, no statistically significant difference was observed between the two groups regarding the mean SWE values (p: 0.133) (Table 1).

The contraction data of non-POP and POP uteri following the application of 10 µL oxytocin were calculated as percentage changes compared to baseline measurements. Accordingly, after 10 µL oxytocin administration, the mean frequency was 110 ±24.49 in the non-POP group and −10±8.29 in the POP group; mean p-p was 31.12±10.64 in the non-POP group and −7.54±6.43 in the POP group; and mean AUC was 80.42±47.68 in the non-POP group and −33.16±10.58 in the POP group. When the measurements were compared, it was found that frequency (p= 0.0002), p-p (p= 0.0082), and AUC (p= 0.0314) values were statistically significantly decreased in the POP group compared to the non-POP group (Figure 1).

Discussion

The results of the present study indicate that while there was no significant difference in the mean ultrasound SWE values between the POP and non-POP groups, women with POP showed a significant decrease in contraction speed, duration, and force compared to women without POP. This finding makes our study original in this aspect.

It is difficult to explain the multifactorial and complex pathophysiology of prolapse with a single mechanism. Smooth muscle (SM) and connective tissue, which are integral parts of the vaginal wall and the endopelvic structures that support the pelvic organs, also play a role in the pathophysiology of POP.[21] In individuals with prolapse, it has been shown that there is a reduction in the amounts of elastin and collagen, changes in collagen subtypes and collagen metabolism, weakening of nerve conduction, and a decrease in SM amounts.[21,22] It has been shown that there is a reduction in SM fraction in the pelvic support tissues of POP patients. One-third of the uterosacral ligament closest to the uterus contains the highest amount of SM fibers, and it has been shown that structural defects and functional loss occur in the SM cells of this region in women with POP.[23] It has also been shown that the SM fraction significantly decreases in the round ligament of women with uterine prolapse in histological sections stained with hematoxylin and eosin and Masson's trichrome and through morphometric analysis.[24]

SM fibers originating from the vaginal wall connect to the levator ani complex. It has been shown that in women with prolapse, the fraction of SM in the muscular tissue of the apex of the anterior and posterior vaginal walls is reduced compared to women without prolapse.[23] In the present study, we also observed a significant decrease in uterine

contractions in women with POP compared to those without POP. This decrease in uterine contractions may be considered a risk factor for development of prolapse.

The uterus is not just a passive organ that contracts during childbirth. It is composed of SMs that show spontaneous rhythmic contractions and relaxations, changing throughout the menstrual cycle. The frequency of uterine contractions increases mid-cycle and decreases during the luteal phase. Retrograde uterine contractions predominate in mid-cycle, facilitating sperm transport, while convergent uterine contractions, dominant during the luteal phase, are thought to ease embryo implantation.[25] It has been shown that uterine activity does not cease after menopause, and although myometrial contractions decrease, they continue during the postmenopausal period.[26,27] As shown in the present study, this decrease in uterine contractility in women with POP could also play a role in the etiology of POP, independent of menopause.

There is strong evidence linking benign hysterectomy to an increased risk of POP. During total hysterectomy, cutting the cardinal and sacrouterine ligaments may lead to loss of pelvic floor support as the upper third of the vagina is deprived of the support of these ligaments.[28] Although subtotal abdominal hysterectomy is predicted to reduce the risk of prolapse posthysterectomy, it has been shown that subtotal hysterectomy does not have a significant protective effect on pelvic floor support when compared to total abdominal hysterectomy.[29,30]

Putting this evidence together, the uterus could theoretically be a component of the pelvic floor support system. Furthermore, decreased uterine contractility in patients with POP may also be a consequence of prolapse. On the other hand, relaxation or impairment of the supportive components of pelvic floor, as well as increased intraabdominal pressure,disruptthe biomechanical integrity of pelvic floor. This alteration consequently leads to changes in the axes, positions and morphological characteristic of the uterus and vagina.[31] Although this study demonstrated a reduced oxytocin response in isolated uterine tissue, changes in uterine position and loss of pelvic support structures in advanced POP cases may also contribute to decreased uterine contractility. Therefore, this reduction may reflect not only a physiological contraction disorder but also the combined effect of structural and positional factors.

After the birth of the fetus, the primary mechanism of uterine hemostasis is the contraction of the myometrium, which mechanically compresses the vessels supplying the placental bed. Uterine atony occurs due to insufficient contraction of the uterine myometrial cells in response to endogenous oxytocin secreted during labor.[32] It has been shown that in women with POP, the incidence of postpartum hemorrhage, uterine inversion, shock, blood product transfusion, and hysterectomy is higher compared to women without POP.[13] One of the causes of pregnancy complications in women with apical prolapse may be reduced uterine contractions; however, this hypothesis requires further investigation.

SWE uses high-frequency ultrasound waves to obtain information about tissue elasticity. In this context, the present study compared the SWE of the myometrium in women with POP with those without POP. When measured using sonoelastography, we could not detect a significant difference in myometrial elasticity between the POP and non-POP groups. In a recent study using transabdominal SWE, the average SWE value in the healthy myometrium of a completely visible uterus was found to be 7.26 kPa.[33] In a previous study we conducted comparing transabdominal SWE measurements of a healthy uterus and uterus with adenomyosis, the average SWE value of a healthy myometrium was found to be 8.1 kPa.[20] The similar values obtained in the present study (POP group 8.52 kPa, non-POP group 10.01 kPa) further support the reference values for normal myometrium in transabdominal SWE measurements.

Egorov et al[34] conducted biomechanical mapping of the female pelvic floor using a vaginal tactile imaging device that records high-resolution pressure patterns along the vaginal walls during pelvic floor muscle contractions. They reported that this method could provide a unique set of parameters that characterize uterine prolapse compared to normal conditions. In their study, they demonstrated a significant reduction in pelvic muscle contraction capacity in uterine prolapse. They suggested that these biomechanical measurements could provide insights into the functional relationships between the patient's support tissues and underlying muscular support, emphasizing that they could be useful in future research and practical applications. The above studies, which show that SM contractility and elasticity may play an important role in the etiology of POP, can shed light on further studies like ours. In the studies mentioned above, pelvic floor muscles were generally the focus of POP evaluation and etiology. The present study differs from other studies in the literature because the uterus was considered as a supporting tissue in POP cases, and uterine contractility was compared with non-POP cases.

To the best of our knowledge, this is the first study to examine and compare isolated uterine contractions and uterine stiffness in POP patients and controls. However, there are certain limitations of our study: it involves a relatively small

sample size, patients are in the pre/postmenopausal period as the study was conducted on hysterectomy materials, and there are no similar studies for data interpretation. The strength of the study is that it was conducted on human uterine tissue, not animal models. This avoids interspecies differences.

Conclusion

In conclusion, a relationship has been identified between reduced uterine contractions and pelvic organ prolapse. The results obtained in the present study suggest that decreased uterine contractility may be a potential risk factor contributing to the onset or progression of POP.

Future studies are needed to determine whether changes in uterine contractility are a cause or a consequence of POP.

Abbreviations

POP, pelvic organ prolapse; PFD, pelvic floor dysfunction;SWE, shear wave elastography; AUC, area under curve; p-p, peak to peak; FOV, field of view; ROI, region of interest; kPa, kilopascal.

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Disclosure

The authors have no conflicts of interest.

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