Archives of Gynecology and Obstetrics

Influence of epidemiological characteristics (age, parity and other factors) in the assessment of healthy uterine cervical stiffness evaluated through shear wave elastography as a prior step to its use in uterine cervical pathology

--Manuscript Draft--

Manuscript Number:	ARCH-D-20-00051R3
Full Title:	Influence of epidemiological characteristics (age, parity and other factors) in the assessment of healthy uterine cervical stiffness evaluated through shear wave elastography as a prior step to its use in uterine cervical pathology
Article Type:	Original Article
Section/Category:	Images in Obstetrics and Gynecology
Keywords:	Elastography; ultrasound; Genital, imaging methods, uterus.
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Funding Information:	
Abstract:	I Purpose: To evaluate stiffness changes occurring in the healthy uterine-cervix according to age, parity, phase of the menstrual-cycle and other factors by shear wave elastography (SWE). Methods: Evaluations of cervical speed and stiffness measurements were performed in 50 non-pregnant patients without gynaecological pathology using SWE transvaginal ultrasound. We performed the evaluation in the midsagittal plane of the uterine-cervix with measurements at 0.5, 1 and 1.5 cm from external cervical os, at both: anterior, posterior cervical lips. Results: We evaluated 44 patients by SWE and obtained a total average velocity of 3.48 ±1.08 m/s and stiffness of 42.39 ±25.33 kPa. We found differences in speed and stiffness according to the cervical lip and depth evaluated; thus, we observed a velocity of 2.70 m/s at 0.5cm of depth in the anterior lip and 3.53 m/s at 1.5 cm of depth in the posterior lip (p<0.05). We observed differences according to parity, obtaining a wave transmission speed of 2.67 m/s and 4.41 m/s at the cervical-canal of nulliparous and multiparous patients, respectively (p 0.002). We observed differences according to patient age (from a speed of 2.75m/s at the cervical canal in the age group of 20-35 years old to 5.05 m/s in the age group >50 years old) (p<0.008). We did not observe differences in speed or stiffness according to the phase of the menstrual-cycle, BMI,

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Running headline: ASSESSMENT OF HEALTHY UTERINE CERVICAL STIFFNESS WITH ELASTOGRAPHY

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Word count of abstract: 246

Word count of main text: 2544

INFLUENCE OF EPIDEMIOLOGICAL CHARACTERISTICS (AGE, PARITY, AND OTHER FACTORS) IN THE ASSESSMENT OF HEALTHY UTERINE CERVICAL STIFFNESS EVALUATED THROUGH SHEAR WAVE ELASTOGRAPHY AS A PRIOR STEP TO ITS USE IN UTERINE CERVICAL PATHOLOGY

Abstract.

Purpose: To evaluate stiffness changes occurring in the healthy uterine-cervix according to age, parity, phase of the menstrual-cycle and other factors by shear wave elastography (SWE).

Methods: Evaluations of cervical speed and stiffness measurements were performed in 50 non-pregnant patients without gynaecological pathology using SWE transvaginal ultrasound. We performed the evaluation in the midsagittal plane of the uterine-cervix with measurements at 0.5, 1 and 1.5 cm from external cervical os, at both: anterior, posterior cervical lips.

Results: We evaluated 44 patients by SWE and obtained a total average velocity of 3.48 ±1.08 m/s and stiffness of 42.39 ±25.33 kPa. We found differences in speed and stiffness according to the cervical lip and depth evaluated; thus, we observed a velocity of 2.70 m/s at 0.5cm of depth in the anterior lip and 3.53 m/s at 1.5 cm of depth in the posterior lip ($p<0.05$). We observed differences according to parity, obtaining a wave transmission speed of 2.67 m/s and 4.41 m/s at the cervical-canal of nulliparous and multiparous patients, respectively (p 0.002). We observed differences according to patient age (from a speed of 2.75m/s at the cervical canal in the age group of 20-35 years old to 5.05 m/s in the age group >50 years old) ($p<0.008$). We did not observe differences in speed or stiffness according to the phase of the menstrual-cycle, BMI, smoking-status or the presence or absence of non HPV infections.

Conclusions: The wave transmission speed and stiffness of the uterine cervix evaluated by SWE varies according to the cervical lip and depth of the evaluation as well as according to the parity and age of the patient.

Main test

INTRODUCTION

Currently, there is an increase in cervical uterine pathology and the incidence of cervical cancer. The clinical management is based on cytologic and colposcopic studies, which are sometimes insufficient for a satisfactory approach to this pathology, necessitating the improvement of diagnostic methods and the development of new technologies (1).
A new research method in ultrasound, namely, sonoelastography, facilitates evaluating the elasticity of tissues (2-7). We know that elasticity is a characteristic of tissues, that it changes during different pathological processes (trauma, inflammation, tumours) and that any new formation with high stiffness is associated with a higher risk of malignancy (8).

Elastography is based on the same principle as palpation. The pressure causes deformation in tissues, and this deformation is higher in soft tissues than in rigid ones. The evaluation of the deformation rate allows for the determination of the elasticity of tissues (2-7). In elastography, pressure is created by light mechanical compression (strain elastography, SE) or by ultrasound waves (shear wave elastography, SWE), and computer software can express the degree of elasticity by different colours (qualitative or semi-qualitative) or by numerical evaluation (quantitative) (3-6,9).

Elastography, which has come to be called the "visual palpation method", is already widely used in different organs, such as the prostate, liver or breast (10-17), but its utility in the field of obstetrics and gynaecology is limited (7,9,18-28). Some authors have begun to use sonoelastography in the evaluation of cervical uterine pathology (1,25) using pressure elastography (strain elastography, SE); however, this type of elastography in the uterine cervix has difficulties because of the lack of surrounding tissue and the inability to reliably quantify, and hence reproduce, the transducer pressure applied to the cervix (7). SWE does not present these limitations and is a promising technique for evaluating the stiffness of the uterine cervix in pathological situations (9). However, it is known that the histological characteristics of the healthy cervix can vary for different reasons, such as parity, age or hormonal influence (29-33). For this reason, we believe it is necessary prior to the use of SWE in the clinical assessment of cervical pathology to assess changes in SWE in the healthy cervix according to different epidemiological variables, such as age, parity, and phase of the menstrual cycle.

MATERIALS AND METHODS

We conducted a cross-sectional observational study with 50 women between December 2017 and April 2018 at the Valme University Hospital.

Subjects

The patients studied were women aged between 18 and 65 years without previous cervical pathology (normal cytology in the last year). In a single visit, the technique to be performed was explained to the patients, and they were invited to participate in the study; a complete gynaecological examination was performed, including transvaginal ultrasound in B mode prior to sonoelastography. Patients with cervical pathology on transvaginal ultrasound in B mode or with uterine pathology (e.g., myoma) or functional or organic adnexal pathology were excluded.

Imaging techniques

The sonoelastography was performed by two operators with more than 5 years of experience in gynaecological ultrasound and with specific training in sonoelastography using a Toshiba Aplio 500 PlatinumTM ultrasound scanner (Canon Medical Systems, Tochigi, Japan) with an 11C3 PVT-781VTE intracavitary transducer. A machine setting of a shear wave frequency of 4 MHz, tracking of 0, was employed. This setting uses a 4-MHz push pulse and 4-MHz tracking pulse. Shear wave speed measurements were obtained using the continuous mode and the lowest frame rate setting of 1, equating to 0.4 frames per second. The elastogram map was stable for at least 3 s before speed measurements were obtained (34,35).

For this procedure, ultrasound gel is placed with the help of a speculum into the vagina to improve the delimitation of the contour of the cervix and the canal and to decrease the pressure exerted on the cervix (36). The probe is placed without any pressure on the cervix and the evaluation by SWE is performed in the midsagittal plane of the uterine cervix, visualizing the cervical canal horizontally, and the cervix occupies three-quarters of the screen. The 30 mm region of interest (ROI) is centred on the study area. The elastogram map opacity was set to 0.3. Utilizing Canon technology, the accuracy of shear wave propagation can be assessed in a number of ways. This elastogram speed map was set to a scale of 0.5 to 8.5 cm/s, with blue being indicative of softer tissues. The non-existence of peripheral red colours, indicative of overpressure, is confirmed, and parallel lines are required in the study area in the wave front propagation map. (Figure 1 and 2). Three measurements were made in each study area by means of a 2 mm circular study window to calculate the mean, standard deviation and median of both the velocity (m / s) of propagation and the elasticity (Kilopascals) of the tissue at 0.5, 1 and 1.5 cm from the external cervical os (distance measurement with caliper prior to evaluation), both in the anterior lip and in the canal and posterior lip of the cervix (Figure 3). In this way, quantitative measurements of the three anatomical regions of study and a qualitative assessment of the entire cervix with a colour map superimposed on the B-mode ultrasound image were obtained (Figure 4). With the first 24 cases, we carried out a double evaluation by patient and by operators to perform an assessment of intraobserver and interobserver concordance.

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics software version 22 (IBM, Armonk, NY). Descriptive statistics were performed using the mean and standard deviation for the numerical variables and percentages for the qualitative variables. The numerical variables were compared by Student’s t-test if the normality of the data was verified (Shapiro-Wilk test) or by the Mann-Whitney test if they were not normal. The qualitative variables were compared by the chi-squared test or Fisher's exact test. P values <0.05 were considered statistically significant. The intraobserver and interobserver concordance was analysed using intraclass correlation coefficients and their 95% confidence intervals.

The study protocol was reviewed and approved by the Ethics Committee of Valme. University Hospital (1001-N-18), and informed consent was obtained from all patients.

RESULTS

Of the 50 women included in the study, sonoelastography was performed in 44. Two cases were excluded due to not having the previous cytology result, and 4 cases were excluded due to uterine or adnexal pathology. We divided the 44 patients studied into 3 groups according to age: from 20 to 34 years (18 women), from 35 to 49 years (14 women) and from 50 to 65 years (12 women). Their epidemiological characteristics are presented in Table 1.

We have observed an intraobserver and interobserver correlation of stiffness and speed of 0.99 (95% CI, 0.95-1.00) in anterior lip at 0.5 cm, 1 cm, cervical canal at 0.5 cm, 1cm and posterior lip at 0.5 cm. The intraobserver and interobserver correlation of stiffness and speed in anterior lip at 1.5 cm is 0.96 (95% CI, 0.87-0.99) and 0.94 (95% CI, 0.78-0.99) respectively. The intraobserver and interobserver correlation of stiffness and speed in cervical canal at 1.5 cm is 0.98 (95% CI, 0.90-0.99) and 0.98 (95% CI, 0.90-0.99) respectively. The intraobserver and interobserver correlation of stiffness and speed in posterior lip at 1 cm and 1.5 cm is 0.97 (95% CI, 0.96-0.99), 0.96 (95% CI, 0.90-0.99) and 0.91 (95% CI, 0.64-0.99), 0.90 (95% CI, 0.60-0.99). respectively.

We obtained a total average wave speed of 3.48 ± 1.08 m/s, with a total average stiffness of 42.39 ± 25.33 kPa. We observed differences in these values in relation to depth and anatomical region (Table 2). Thus, we showed differences in speed within the anterior lip, ranging from 2.70 m/s at 0.5 cm to 3.00 m/s at 1.5 cm ($p < 0.05$). Similarly, we found an average velocity of 2.90 m/s at 1 cm in the anterior lip and 3.10 m/s at 1 cm in the posterior lip ($p < 0.05$). Similar results occur in relation to stiffness.

We found significant differences in most of the cervical anatomical regions when comparing the speed of transmission and stiffness according to parity, with a higher speed of wave transmission and stiffness in multiparous women versus primiparous and nulliparous women (from a speed of 2.63 m/s at the cervical canal of nulliparous women to 4.41 m/s at the cervical canal of multiparous women) ($p < 0.002$) (Table 2 and Figure 4).

We also found significant differences in most of the anatomical study areas in the different age groups, reaching higher values in both speed and stiffness in the age group of 50-65 years (obtaining a speed of 2.75 m/s at the cervical canal in the age group of 20-35 years and 5.05 m/s in the group > 50 years old) ($p<0.008$) (Table 3).

We did not observe differences in transmission speed or stiffness in relation to BMI (Table 4), the influence of smoking (Table 4), the presence of non-HPV infection (Table 5) or the phase of the menstrual cycle (Table 5).

DISCUSSION

The main finding in our study is that in assessing the rigidity of the uterine cervix by SWE in non pregnant patients, both the location and depth of the evaluation point as well as the maternal characteristics of age and parity should be taken into account. In addition, physiologically, the uterine cervix of a postmenopausal multiparous woman at the deep level of the posterior lip presents greater rigidity, and the less rigid cervix is that of a young nulligravid woman at the superficial level of the anterior lip.

Elastography was described more than 20 years ago as an important complement to ultrasound. Elastography is a non-invasive method that uses images of soft tissue stiffness to detect or classify masses that are more rigid than the tissue in which they settle, consequently acquiring a special utility for the evaluation of suspicious lesions in the prostate, thyroid or breast (10-17). Perhaps the most widespread utility of this technique is its application for the quantitative determination of the degree of hepatic fibrosis (Fibroscan®), replacing in some cases the need for a liver biopsy in patients with cirrhosis (37).

Elastography is classified according to the principles of stress elastography (strain elastography, SE) and wave elastography (shear wave elastography, SWE), depending on whether the impulse source is generated by external pressure or by internal artificial force. SE provides images that describe the extent of tissue deformation under pressure from an external impulse, measuring the displacement of tissue in a very short time interval. A large displacement is characteristic of the soft tissues, and hard tissues are characterized by a small displacement. The results are expressed qualitatively with colours (blue, soft: red, hard; and green, medium hard) or semiquantitatively (by the percentage of colours or by the strain ratio); however, SE cannot determine the absolute value of tissue stiffness.
In contrast, SWE uses an ultrasonic wave to generate an artificial impulse, which propagates a transverse wave (shear wave) through the tissue. When this wave passes through the tissue, its speed varies depending on its rigidity, which allows the measurement of its stiffness (in kPa) or speed of propagation (in m/s) (2-6,9). SWE is a quantitative method to assess tissue stiffness, which is also an independent operator (38). These are the reasons it is preferable to evaluate the stiffness of the uterine cervix by SWE rather than by SE.

In gynaecology and obstetrics, the use of elastography in recent years has been multiplied. During gestation, its use has focused on the qualitative evaluation (SE) of stiffness for the study of preterm delivery and for assessing the induction of labour (19,27,28), although there are already studies using SWE (26,29). In reproductive medicine, the use of elastography has begun (39,40), and in gynaecology, it is currently being used to differentiate myometrial pathologies (myomas versus adenomyosis) and endometrial pathologies (polyps versus endometrial cancer) and for their management (41-44), but cervical uterine pathology is where this new ultrasound technique has been used in recent years.

Lu et al. (45) tried to differentiate between benign and malignant uterine cervical pathology by SE and identified a malignancy cut-off point of 4.52 (strain ratio) (sensitivity 90.9%, specificity 90.0%, positive predictive value 90.5% and negative predictive value 90.9%). Sun et al. (46) reported a strain ratio of malignant lesions of 8.19 versus 2.81 in benign lesions. Xu et al. (47) also used SE imaging to evaluate the response in cases of locally advanced cervical cancer to chemo-radiotherapy. Ma et al. (48) used this technique with good results to evaluate parametrial infiltration in cases of cervical cancer.
Such studies have been performed by SE, and the limitation of this technique to assess stiffness in the uterine cervix is known due to its difficulty in standardization. Consequently, O'Hara et al. (35) used SWE to evaluate the uterine cervix and proposed identifying the biological and technical confounding factors for evaluating the uterine cervix by SWE. These authors note that the evaluation of the anterior cervix lip by SWE is adequate and reproducible but that the evaluation of the posterior cervix by SWE is limited. The authors identify that the pressure exerted on the cervix can influence the speed of transmission of

the wave. However, they assure that by controlling this aspect and standardizing the technique, the evaluation of the uterine cervix by SWE is possible.

Firstly we observe is that the stiffness and speed evaluated by SWE present a good intra and inter-observer correlation. Also we observed by SWE that, when a quantitative evaluation is performed, the stiffness of the healthy uterine cervix varies according to the location and depth of where it is evaluated as well as according to parity and age.

The cervix is composed of epithelial tissue associated with glandular tissue and a thick stroma rich in water and proteins of the extracellular matrix (to a greater extent, collagen, elastin, glycosaminoglycans, hyaluronic acid and dermatan sulfate and, to a lesser extent, fibronectin and smooth muscle) (29,30). The composition of this extracellular matrix changes during pregnancy but also with age, parity and hormonal status. Therefore, it is known that in the face of the changes that this extracellular matrix may undergo in relation to the amount of water and proteins, its rigidity changes, and our study observed these rigidity changes through elastography (31-33). Consequently, we believe that to evaluate uterine cervical pathology by elastography, one must take into account in which cervical lip and at what depth the lesion is being evaluated, as well as the epidemiological characteristics of patient age and parity.

We consider one limitation of our study to be that: our study contains a quiet small number of cases, especially for the subgroup analysis, we evaluated the cervix exclusively in the horizontal position because we believe that the SWE evaluation should also be performed in other positions (posterior, vertical and angulated). We also regard our use of a wide study window (30 mm) as a limitation because with a smaller study window (15-20 mm), artefacts can be minimized and the SW technology is vulnerable to measurement errors, especially in the near field region of the ultrasound probe.

Conclusion: The wave transmission speed and stiffness of the uterine cervix evaluated by SWE varies according to the cervical lip and depth of the evaluation as well as according to the parity and age of the patient.

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Table 1. Epidemiological characteristics of study population.

Age range	Total		44 (100%)	20-34		18 (40.9%)	35-49		14 (31.8%)	50-65		12 (27.2%)
Age	38.98 ± 13.66			25.44 ± 3.22			40.5 ± 3.29			57.5 ± 4.9		
Body Mass Index	23.87 ± 3.64			21.57 ± 1.82			25.21 ± 3.85			25.76 ± 3.78		
Smoker	**Yes**	**No**		**Yes**	**No**		**Yes**	**No**		**Yes**	**No**	
	12 (27.3%)	32 (72.7%)		5 (41.7%)	13 (27.8%)		6 (42.9%)	8 (57.1%)		1 (8.3%)	11 (91.7%)	
Vaginal deliveries	**Nulliparous**	**Primiparous**	**Multiparous**	**Nulliparous**	**Primiparous**	**Multiparous**	**Nulliparous**	**Primiparous**	**Multiparous**	**Nulliparous**	**Primiparous**	**Multiparous**
	23 (52.3%)	4 (9.1%)	17 (38.6%)	17 (94.4%)	0 (0%)	1 (5.6%)	5 (35.7%)	3 (21.4%)	6 (42.9%)	1 (8.3%)	1 (8.3%)	10 (83.3%)
Menstrual cycle phase	**Amenorrhea**	**1st phase**	**2nd phase**	**Amenorrhea**	**1st phase**	**2nd phase**	**Amenorrhea**	**1st phase**	**2nd phase**	**Amenorrhea**	**1st phase**	**2nd phase**
	15 (34.1%)	12 (27.3%)	17 (38.6%)	1 (5.6%)	7 (38.9%)	10 (55.6%)	3 (21.4%)	5 (35.7%)	6 (42.9%)	11 (91.7%)	0 (0%)	1 (8.3%)
Menopause	**Yes**	**No**		**Yes**	**No**		**Yes**	**No**		**Yes**	**No**	
	11 (25%)	33 (75%)		0 (0%)	18 (100%)		0 (0%)	14 (100%)		11 (91.7%)	1 (8.3%)	
Cervical cytology	**Normal**	**Infection**		**Normal**	**Infection**		**Normal**	**Infection**		**Normal**	**Infection**	
	38 (86.4%)	6 (13.7%)		16 (88.9%)	2 (11.2%)		11 (78.6%)	3 (21.4%)		11 (91.7%)	1 (8.3%)	

Table 2. Cervical Uterine Shear Wave Elastography (SWE). Wave transmission speed (m/s) and stiffness (kPa) in the different anatomic study areas according to vaginal deliveries.

p1: level of statistical significance (p) of the differences in speed between different groups of the study population.

p2: level of statistical significance (p) of the differences in stiffness between different groups of the study population.

Vaginal deliveries									
		Nulliparous n=23		Primiparous n=4		Multiparous n=17			
		Speed	Stiffness	Speed	Stiffness	Speed	Stiffness	p1	p2
Anterior lip	0.5 cm	2.70 ± 1.04	26.48 ± 23.50	2.61 ± 1.02	24.67 ± 19.09	3.42 ± 1.74	46.34 ± 48.55	0.490	0.490
	1 cm	2.90 ± 0.82	28.41 ± 17.66	3.25 ± 1.19	36.40 ± 23.44	3.76 ± 1.50	49.67 ± 39.02	0.190	0.230
	1.5 cm	3.07 ± 0.97	33.10 ± 22.29	3.71 ± 1.18	48.92 ± 37.96	4.43 ± 1.74	65.77 ± 47.60	0.040	0.410
Cervical canal	0.5 cm	2.60 ± 1.00	25.14 ± 24.88	3.17 ± 2.95	42.42 ± 64.44	3.72 ± 1.79	46.36 ± 37.26	0.060	0.100
	1 cm	2.63 ± 0.73	23.60 ± 13.02	3.83 ± 2.53	32.27 ± 21.74	4.41 ± 1.77	61.49 ± 38.38	0.002	0.002
	1.5 cm	2.95 ± 0.84	30.37 ± 16.60	3.97 ± 2.12	52.45 ± 50.63	4.62 ± 1.33	67.99 ± 37.14	0.012	0.002
Posterior lip	0.5 cm	2.89 ± 0.98	30.17 ± 22.17	2.27 ± 1.00	19.87 ± 17.73	3.44 ± 1.09	41.65 ± 24.61	0.061	0.058
	1 cm	3.10 ± 0.64	32.40 ± 11.88	3.28 ± 1.11	38.65 ± 25.42	3.99 ± 1.11	55.46 ± 28.32	0.040	0.057
	1.5 cm	3.53 ± 0.93	39.15 ± 17.08	3.74 ± 2.47	31.47 ± 19.82	4.42 ± 1.64	73.28 ± 35.82	0.258	0.001
Total		3.03 ± 0.6	31.89 ± 12.42	3.40 ± 1.38	37.25 ± 24.61	4.14 ± 1.25	58.47 ± 31.43	0.051	0.055

Table 3. Cervical Uterine Shear Wave Elastography (SWE). Wave transmission speed (m/s) and stiffness (kPa) in the different anatomical areas of study according to age range.

p1: level of statistical significance (p) of the differences in speed between different groups of the study population.

p2: level of statistical significance (p) of the differences in stiffness between different groups of the study population.

Age range									
		20-34 n=18		35-49 n=14		50-65 n=12			
		Speed	Stiffness	Speed	Stiffness	Speed	Stiffness	p1	p2
Anterior lip	0.5 cm	2.64 ± 1.12	26.17 ± 25.71	2.46 ± 0.72	20.75 ± 12.73	4.06 ± 1.75	61.13 ± 51.93	0.009	0.013
	1 cm	2.97 ± 0.87	30.17 ± 19.15	3.01 ± 0.97	30.68 ± 19.32	4.02 ± 1.62	56.64 ± 43.49	0.169	0.235
	1.5 cm	3.20 ± 1.05	36.36 ± 24.23	3.52 ± 1.20	40.60 ± 27.48	4.51 ± 1.95	71.78 ± 54.94	0.226	0.278
Cervical canal	0.5 cm	2.51 ± 1.13	24.66 ± 28.28	2.52 ± 0.72	21.75 ± 11.13	4.79 ± 2.02	69.45 ± 45.51	0.003	0.004
	1 cm	2.75 ± 0.78	25.50 ± 14.17	2.96 ± 1.14	31.65 ± 25.39	5.05 ± 2.03	68.68 ± 39.27	0.008	0.008
	1.5 cm	3.03 ± 0.92	32.24 ± 17.96	3.47 ± 1.10	40.74 ± 26.27	5.01 ± 1.63	77.93 ± 44.28	0.009	0.007
Posterior lip	0.5 cm	2.92 ± 0.91	30.25 ± 21.32	2.54 ± 0.94	24.16 ± 18.99	3.87 ± 1.02	50.64 ± 24.09	0.004	0.004
	1 cm	3.05 ± 0.68	31.71 ± 12.72	3.18 ± 0.62	34.48 ± 14.04	4.46 ± 1.02	66.64 ± 27.43	<0.0005	0.003
	1.5 cm	3.59 ± 0.94	39.43 ± 15.07	3.66 ± 1.56	51.02 ± 32.10	4.65 ± 1.76	70.19 ± 39.91	0.246	0.108
Total		3.07 ± 0.66	32.89 ± 13.47	3.32 ± 0.86	38.2 ± 19.79	4.40 ± 1.41	64.00 ± 34.96	0.058	0.086

Table 4. Cervical Uterine Shear Wave Elastography (SWE). Wave transmission speed (m/s) and stiffness (kPa) in the different anatomical areas of study according to body mass index (BMI) and to smoking habit.

In body mass index (BMI)

p1: level of statistical significance (p) of the differences in speed between different groups of the study population.

p2: level of statistical significance (p) of the differences in stiffness between different groups of the study population.

In smoking habit

p3: level of statistical significance (p) of the differences in speed between different groups of the study population.

p4: level of statistical significance (p) of the differences in stiffness between different groups of the study population.

	Body Mass Index						Smoker							
		Normal Weight n=28		Overweight-Obesity n=16			Yes n=12		No n=32					
		Speed	**Stiffness**	**Speed**	**Stiffness**		**Speed**	**Stiffness**	**Speed**	**Stiffness**	**p1**	**p2**	**p3**	**p4**
Anterior lip	**0.5 cm**	3.04 ± 1.55	37.00 ± 41.58	2.88 ± 1.08	19.37 ± 24.56		3.41 ± 1.72	45.45 ± 47.99	2.83 ± 1.24	30.28 ± 30.93	0.792	0.870	0.209	0.209
	1 cm	3.44 ± 1.32	42.15 ± 33.63	2.93 ± 0.90	28.49 ± 16.94		3.48 ± 1.27	42.42 ± 28.58	3.18 ± 1.91	35.44 ± 29.81	0.217	0.203	0.396	0.322
	1.5 cm	3.77 ± 1.50	50.70 ± 40.20	3.44 ± 1.39	40.49 ± 33.39		3.85 ± 1.80	53.32 ± 46.20	3.58 ± 1.33	44.82 ± 34.98	0.312	0.282	0.800	0.412
Cervical canal	**0.5 cm**	3.03 ± 1.79	34.19 ± 37.44	3.14 ± 1.23	35.37 ± 31.28		3.52 ± 1.56	44.71 ± 40.91	2.90 ± 1.62	30.88 ± 32.64	0.320	0.347	0.085	0.097
	1 cm	3.44 ± 1.85	38.57 ± 34.15	3.38 ± 1.13	39.39 ± 27.04		3.34 ± 1.81	35.88 ± 30.48	3.45 ± 1.57	39.92 ± 32.21	0.520	0.470	0.756	0.866
	1.5 cm	3.66 ± 1.53	46.47 ± 36.28	3.66 ± 1.17	45.94 ± 29.56		3.64 ± 1.68	46.79 ± 42.66	3.67 ± 1.32	46.10 ± 30.72	0.946	0.839	0.632	0.632
Posterior lip	**0.5 cm**	3.13 ± 1.22	35.62 ± 27.26	2.89 ± 0.72	29.87 ± 14.03		3.40 ± 1.36	42.56 ± 30.73	2.91 ± 0.93	30.37 ± 19.79	0.937	0.990	0.350	0.336
	1 cm	3.30 ± 0.96	37.87 ± 21.06	3.75 ± 0.94	48.83 ± 25.66		3.42 ± 0.68	39.22 ± 15.36	3.47 ± 1.06	42.69 ± 25.46	0.194	0.125	0.933	0.883
	1.5 cm	3.91 ± 1.31	52.03 ± 30.67	3.81 ± 1.70	48.69 ± 31.33		3.89 ± 1.83	51.50 ± 41.20	3.87 ± 1.29	50.66 ± 26.48	0.946	0.596	0.974	0.571

		3.51 ± 1.15	43.54 ± 27.09	3.42 ± 0.96	40.02 ± 22.07		3.67 ± 1.22	47.23 ± 30.28	3.41 ± 1.03	40.56 ± 23.53	0.842	0.669	0.455	0.385
	0.5 cm	3.04 ± 1.55	37.00 ± 41.58	2.88 ± 1.08	19.37 ± 24.56		3.41 ± 1.72	45.45 ± 47.99	2.83 ± 1.24	30.28 ± 30.93	0.792	0.870	0.209	0.209
Total		3.44 ± 1.32	42.15 ± 33.63	2.93 ± 0.90	28.49 ± 16.94		3.48 ± 1.27	42.42 ± 28.58	3.18 ± 1.91	35.44 ± 29.81	0.217	0.203	0.396	0.322

Figure 1. A. Sagittal section of the uterine cervix in mode B.

B.1.Shear wave elastography (SWE) of the uterine cervix.B.2. Parallel lines are required in the study area in the wave front propagation map.

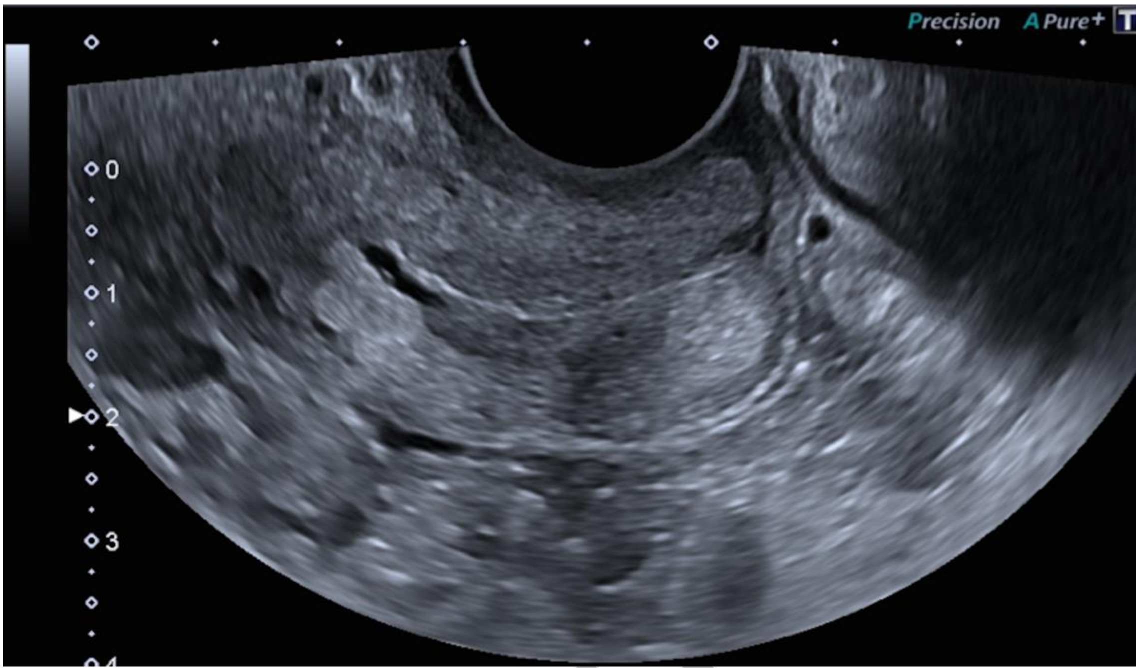

Figure 2. Shear wave elastography (SWE) indicative of overpressure. Peripheral red colours and non-parallel lines in the study area in the wave front propagation map.

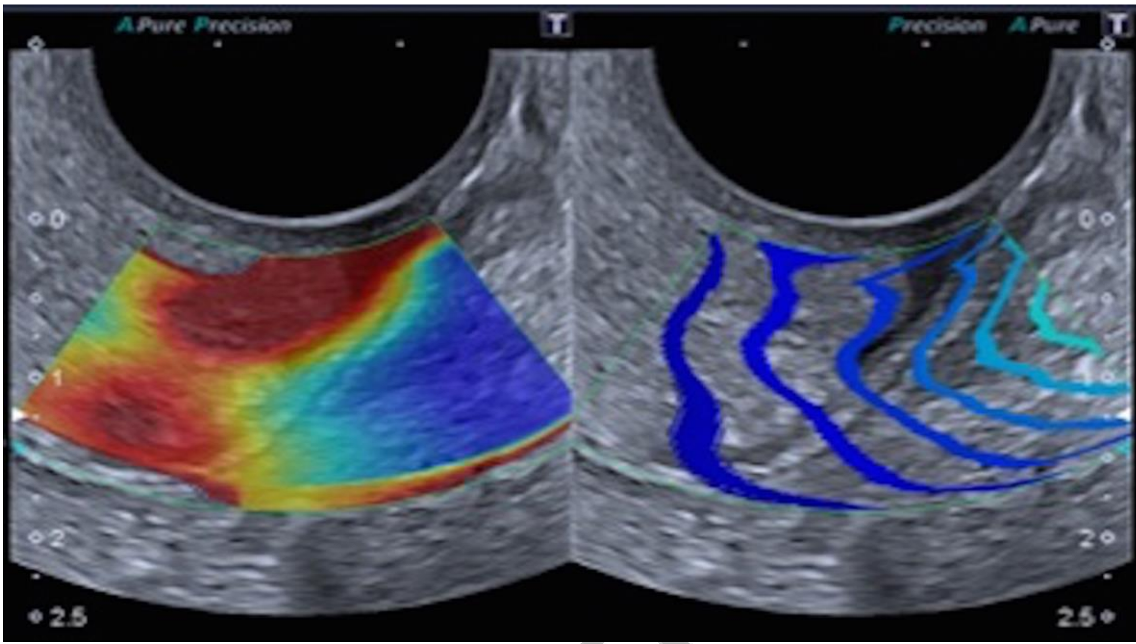

Figure 3. Sagittal section of the uterine cervix. Graphic representation of the study points in the Shear wave elastography (SWE)

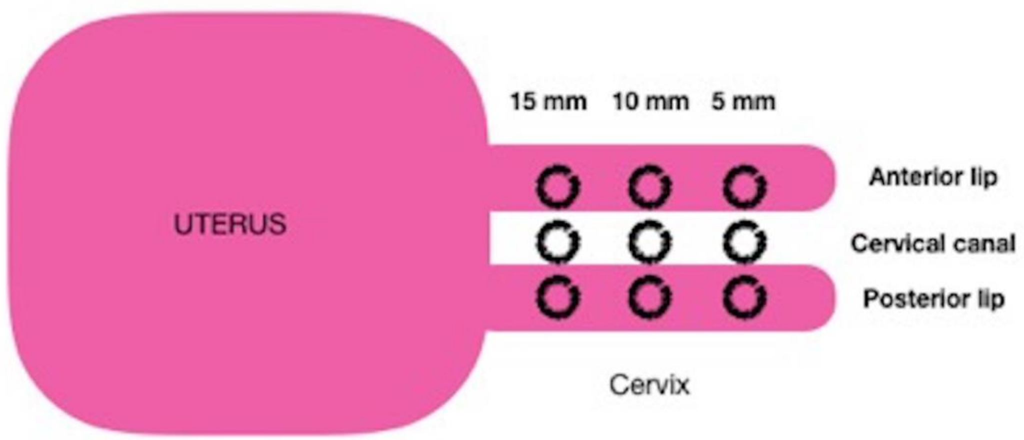

Figure 4. Shear wave elastography (SWE) evaluation of uterine cervix with quantitative measurement of wave propagation stiffness and speed at 0.5 cm (A), 1 cm (B) and 1.5 cm (C) in the anterior lip, cervical canal and posterior lip.