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Original Research

Reliability and agreement of three-dimensional/four-dimensional transperineal ultrasound in women with chronic pelvic pain

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Background: Chronic pelvic pain is a common complaint in the gynecological office. The association among anus levator muscle injury, CPP of unknown origin in parous women, and pelvic sensory symptoms have been demonstrated. The study's purpose is to assess the intrarater/interrater reliability and agreement of pelvic floor biometry and levator ani muscle injury evaluated using three-dimensional ultrasound in women with chronic pelvic pain. *Methods*: Two raters independently and blindly acquired three datasets of three-dimensional transperineal ultrasound volumes. The datasets were evaluated 60 days apart. To assess levator ani muscle injury, the hiatal area/diameter, levator ani muscle thickness, urethra-anus distance, and levator-urethra gap were measured. The intrarater reproducibility and interrater reproducibility were calculated. The concordance correlation coefficients and limits of agreement were analyzed in 147 three-dimensional ultrasound volumes obtained from 49 patients. *Results*: Levator ani muscle injury was detected in 10.2% (n = 5/49), with a good intrarater concordance correlation of >0.90 for anteroposterior diameter, hiatal area, levator-urethra gap, and urethra-anus distance. The hiatal transverse diameter and levator ani muscle thickness presented poor correlation, with limits of agreement of 28.2% and 29.7%, respectively. The levator-urethra gap also presented poor interrater concordance. Overall, the interrater evaluation had moderate to substantial concordance. *Discussion*: In the detection of levator ani muscle injury in parous women, the hiatal anteroposterior diameter, hiatal area, and urethra-anus distance can be reliably assessed using three-dimensional transperineal ultrasound of the pelvic floor. However, owing to poor reliability, the hiatal transverse diameter, levator ani muscle thickness, and levator ani muscle-urethra gap require more studies before they can be applied clinically.

Keywords

Chronic pelvic pain; Dyspareunia; Endovaginal ultrasound; Pelvic floor injury; Reproducibility; Tridimensional ultrasound

1. Introduction

The advent of three-dimensional/four-dimensional (3D/4D) ultrasonography has enabled the evaluation of the pelvic floor in a dynamic, simple manner, with low cost, few contraindications, and less discomfort for patients [1]. The 3D/4D ultrasonography method is suitable for evaluating the integrity and function of the deep layer of pelvic floor muscles (PFMs). It detects changes in the anorectal angle and hiatus size, probably caused by the contraction and relaxation of the puborectalis muscle. Dislocation of the pelvic floor and levator plate can also be estimated, which is related to the contraction and relaxation of the pubococcygeus, iliococcygeus, and ischiococcygeus muscles [2]. Pelvic floor 3D/4D ultrasonography can show the morphometric modifications of the deep layer of PFMs in patients with provoked vestibulodynia associated with an increase in PFM tone and reduced PFM strength and control [3].

Chronic pelvic pain (CPP) affects approximately 4% of the population of women of reproductive age [4]. Developing countries such as Brazil have a higher prevalence, reaching 19% in some studies [5]. Up to 39% of the complaints of women in the primary care unit are related to CPP [6]. It accounts for 40–50% of gynecological laparoscopies, 10% of gynecological consultations, and 12% of hysterectomies [7]. Several diseases are associated with CPP, including irritable bowel syndrome, cystitis, painful bladder syndrome, endometriosis, pelvic inflammatory disease, neuropathic diseases, and musculoskeletal and mental disorders [8].

Previous studies have demonstrated the association among anus levator muscle injury, CPP of unknown origin in parous women [9], and sensory pelvic symptoms [10]. More than 50% of affected women present tenderness of PFMs, associated with higher depression scores [11] and moderate to severe dyspareunia [12]. Despite the possible involvement of the pelvic floor as a primary cause of CPP [13], many women with CPP present symptoms due to secondary involvement of PFMs through neurogenic inflammation or cross-sensitization [14]. Many theories have been proposed, including possible neurovascular and myofascial injuries [9, 15].

Table 1. Characterization of the studied population.

Age	<30	≥30 <40	≥40 <50		
	5 (10.%)	27 (55.1%)	17 (34.9%)		
Self determinaton color	White	Black			
	34 (69.4%)	15 (30.6%)			
BMI	<18.5	≥18.5–24.9	≥25.0–29.9	≥30.0–39.9	≥40.0
	0 (0%)	15 (30.6%)	19 (38.7%)	15 (30.6%)	0 (0%)
Parity	0	1	1–2	>3	
	22 (44.9%)	11 (22.4%)	12 (24.5%)	4 (8.2%)	
Delivey interventions	Vaginal only	C-section only	Both	Episiotomy	
	10 (37%)	8 (29.7%)	9 (33.3%)	8 (16.3%)	
Pain time length (months)	<12	12–24	24–36	36–48	>48
	1 (2.0%)	6 (12.2%)	8 (16.3%)	3 (6.1%)	31 (63.3%)
VAS (mm)	Mild 0–20	Moderate 30–70	Severe 80–100		
	0	14 (28.6%)	35 (71.4%)		

Notes: VAS, Visual analog scale; BMI, Body Mass Index.

Several 3D/4D ultrasonography-based studies showed considerable inconsistencies, such as heterogeneity of samples, large variability in the mode of image acquisition, differences in the evaluated anatomical parameters, and weak reliability and accuracy criteria. Reliability and accuracy are essential elements in the development and use of diagnostic tools, in addition to ensuring quality in clinical studies [16, 17].

Measurement errors can considerably affect the statistical analysis and interpretation. Therefore, it is essential to quantify the error magnitude by calculating the reliability coefficient and assessing its precision. The International Continence Society Clinical Assessment Group recommends performing test-retest and intrarater/interrater reliability evaluations for ultrasound measurements of PFMs [18].

The aims of this study were to verify the intrarater/interrater reliability and agreement of transperineal 3D/4D ultrasonography to identify injuries, and to describe ultrasound morphological parameters in women with CPP dyspareunia without any apparent cause.

2. Patients and methods

2.1 Study design and recruitment of subjects

This study was designed as a prospective observational cross-sectional reliability and agreement study. Forty-nine subjects were consecutively recruited from March 2014 to October 2015 in a referral center (Center for Gynecological Endoscopy and Pelvic Pain) within a tertiary teaching hospital. The characteristics of the study population are summarized in Table 1.

Women of any parity with CPP, with at least 6 months of dyspareunia without an apparent cause, were considered eligible for inclusion. Women with a postmenopausal status, age <18 or >50 years, body mass index >40 kg/m^2, urinary or fecal incontinence, history of perineal surgeries, and history of hysterectomy were not eligible for inclusion in the study. Women with any other cause of CPP (e.g., laparoscopic, histologic, or clinical diagnosis of endometriosis; bladder pain syndrome; and irritable bowel syndrome [Rome III criteria]) or those with clinical signs typical of neuropathic pain were also not eligible [19].

The research protocol included clinical evaluation by a consultant gynecologist, pelvic and abdominal ultrasonography, and pain intensity evaluation using the visual analog scale.

2.2 Ultrasound assessment protocol

2.2.1 Ultrasound 3D volume acquisition

The ultrasound assessments were performed by two raters (rater 1 [MAMVC] and rater 2 [FAAM]) who had training on the 3D/4D ultrasonography methods of pelvic floor evaluation, as previously described by Dietz *et al.* [20]. The raters were blinded to clinical information and independently performed the evaluations using the same equipment (Voluson E8 Expert; GE, General Electric, Milwaukee, WI), with a GE RIC5-9-D 2D/3D/4D microconvex endocavity transducer probe at a frequency range of 4–9 MHz.

Before the ultrasound examinations in the lithotomy position, the patients emptied their bladder. Three transperineal rendered 3D volumes were obtained at rest. The probe was positioned translabially along the sagittal plane. The acquisition angle was set at 120° and included the entire hiatus of the levator ani muscle (LAM), as well as the pubic symphysis, urethra, vagina, paravaginal tissues, rectum, and puborectalis bundle at the white line up to the posterior margin of the anorectal junction. The examiners performed 3D volume acquisition in two preestablished sequences, alternately between patients: sequence 1 (rater 1, rater 2, rater 1) and sequence 2 (rater 2, rater 1, rater 2).

All 3D volumes were included in the analysis. Between the acquisitions of the 3D volumes, the patient stood up, waited a few seconds, and returned to the examination position. This design aimed to reduce the risks of postural interference and the raters' memory effect.

2.2.2 Analysis of 3D volumes

All rendered volumes obtained were backed up for later analysis. Rater 1 (MAVC) and rater 2 (FAAM), who were experienced with 3D volume images, analyzed the 3D volumes randomly while blinded to patient information (patient identifications were omitted in the device). We performed the interrater reliability evaluation in a single moment. For the intrarater reliability evaluation, we analyzed the 3D volumes with an interval of 90 days.

For the measurements, the multiplanar image mode was used, with the images acquired at the midsagittal plane of the section representative of the minimum distance between the posterior edge of the pubic symphysis and the anterior edge of the levator plate posterior to the anorectal angle [20]. We used tomographic ultrasound imaging as the gold standard method for evaluating levator injury. After ensuring proper alignment in the various planes, 3D rendering was turned on, adjusted to a render box thickness of 10.0–20.0 mm and an interslice interval of 1.0–2.5 mm, with the plane of the minimum dimensions included in the region of interest. In the axial plane, the following aspects were measured: hiatal anteroposterior diameter, hiatal transverse diameter, hiatal area render, LAM thickness (3 and 9 o'clock positions), urethra-anus distance, levator-urethra gap (LUG), and levator-pubic bone gap in the presence of LAM avulsion.

The presence of LAM avulsion can be reported by describing its presence/absence or by using a weighting score corresponding to the following [20, 21]: (1) without any lesion, (2) $<$50% muscle injury, (3) partial avulsion $\geq$50%, and (4) complete avulsion of the muscle to the pubic bone. For our purpose, we defined LAM avulsion as the presence of muscle detachment to the pubic bone.

The OmniView software (GE, General Electric, Milwaukee, WI) is an option for performing a multiplanar evaluation. OmniView is a 3D ultrasound application used to reformat anatomy using different cuts such as lines, curved lines, and polyline. The anatomy then reformatted is displayed in multiplanar view in the usual 3D orthogonal plane plus the volume reconstructed in the fourth slide on the screen's bottom right. Volume contrast imaging is an ultrasound application used to reduced acoustic noise and thus increasing image quality. In addition, the width of the slice e.g., 2 or 3 or more mm can be selected by the sonographer in respect to the appropriate thickness. It was used in association with OmniView technique for dynamic volume acquisition and analysis, and interchangeably with the rendering mode for assessing the pelvic hiatal area [22] (Fig. 1).

2.3 Statistical analysis

The sample size was estimated using the Power Analysis and Sample Size (PASS) software (version 11.0.7; PASS, NCSS, LLC, 329 North 1000 East Kaysville, Utah 84037 USA). The statistical test used a one-sided z-test with a 0.05 significance level and power of 80%. Further parameters were considered under a null or alternative hypothesis: ρ (correlation between measurements) of 0.970 and 0.975, υ (relative bias) of 0.150 and 0.050, and ω (ratio of standard deviations) of 1.150 and 1.050. Thus, a sample of at least 40 subjects was adequate.

The data were stored in a database (Microsoft® Excel® 2013) and analyzed using R Studio software (version 0.99.903 for Mac OS). We evaluated the reliability of the measurements obtained in 3D/4D ultrasonography volumes by analyzing the intrarater concordance and interrater concordance. The intrarater concordance was analyzed using measurements at two distinct moments by the same rater. The interrater concordance was analyzed using the first measurements of rater 1 and rater 2.

The concordance correlation coefficient (CCC) and corresponding 95% confidence intervals were calculated for both concordance analysis steps using the epiR package, and interpreted according to the following scoring system: $<$0.90 (poor), 0.90–0.95 (moderate), 0.95–0.99 (substantial), and $>$0.99 (almost perfect). Subsequently, to calculate the limits of agreement (LoAs) of the intrarater and interrater measurements, we used the Bland-Altman analysis (MethComp package for R) [23]. Thereafter, we calculated the mean difference (Δ) and the standard deviation of the difference (SDd) between the two measures and the LoA obtained using the following formulas: LoA (lower) = Δ - (1.96 $\times$ SDd) and LoA (upper) = Δ + (1.96 $\times$ SDd). For the clinical purpose, the reliability/agreement was interpreted using the relative difference between measurements (i.e., a percentage) instead of the absolute difference, as suggested by Martins and Nastri [24], according to the following criteria: CCC $<$ 70%/LoA $>$ 50% (very poor), CCC 70–90%/LoA 20–50% (poor), CCC 90–95%/LoA 10–20% (moderate), CCC 95–99%/LoA 5–10% (good), and CCC $>$ 99%/LoA $<$ 5% (very good).

3. Results

The prevalence of LAM injury in this study was 10.2% (n = 5/49). The parous group had a prevalence of 18.5% (n = 5/27). Despite the small number of subjects, a good intrarater concordance of 0.96/9.6 and 0.89/10.7 (CCC 95–99%/LoA 5–10% [good]) and a moderate interrater concordance of 0.94 and 0.78 (CCC 90–95%/LoA 10–20% [moderate]) were obtained for right and left LUG, respectively. Of these five patients, four have had a vaginal delivery and two have had an episiotomy. All identified lesions were on the right side.

The intrarater analysis showed a CCC of $>$0.90 for anteroposterior diameter, hiatal area, LUG, and urethra-anus distance, with an LoA of $<$10%. Overall, the hiatal transverse diameter and LAM thickness (at 3 and 9 o'clock positions) presented poor correlation (CCC 70–90%/LoA 20–50% [poor]): CCC = 0.85, 0.58 (at 3 o'clock position), and 0.53 (at the 9 o'clock position), with LoAs of 9.6%, 28.2%, and 29.7%, respectively. After excluding the levator ani injury cases, we observed an improvement of CCC for these measures (0.83, 0.61, and 0.57, respectively), although without improvement of the corresponding LoA (Table 2).

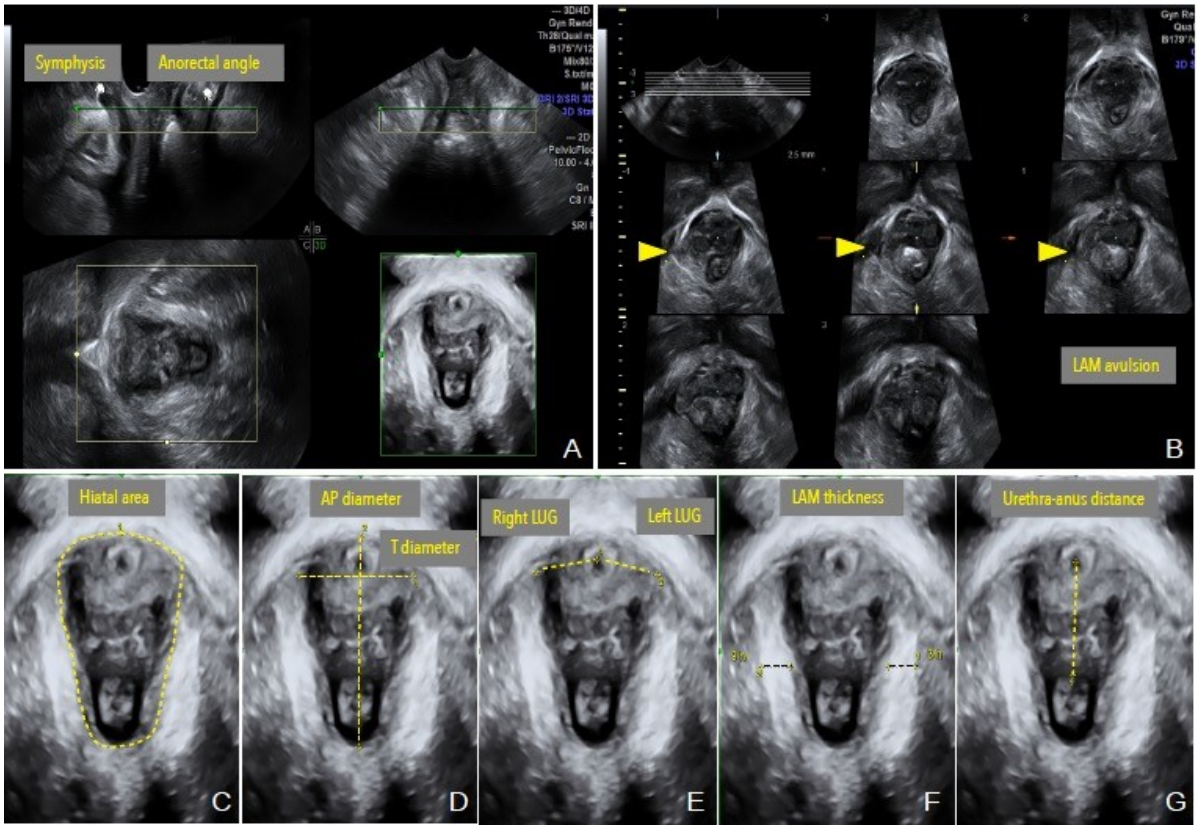

Fig. 1. The rendering technique of three-dimensional ultrasound pelvic floor. (A) Determination of the plane of minimum hiatal dimensions. (B) Imaging of tomographic ultrasound showing total rupture of the levator ani muscle to the right, arrow (score 4). (C) Hiatal area render. (D) Hiatal anteroposterior and transverse diameters. (E) Levator-urethral gap (LUG). (F) Thickness of the levator ani muscle (LAM) at 3 and 9 hours. (G) Distance between the urethra and the upper edge of the anus.

The interrater analysis showed moderate to substantial concordance, except for the hiatal transverse diameter, LAM thickness (at 3 and 9 o'clock positions), and LUG, which presented weak interrater CCC (CCC $<$ 70%/LoA $>$ 50% [very poor]) of 0.62, 0.30, 0.32, and 0.78, respectively, with LoA $>$ 15%. In intraobserver analysis, after excluding the LAM injury cases, we observed an improvement of CCC for these measures, to 0.67, 0.45, 0.39, and 0.66, respectively, although without improvement of the corresponding LoA (Table 3).

4. Discussion

Our findings indicated that transperineal ultrasonography in women with CPP, despite not evaluating the pain intensity, has good intrarater and interrater reliability for measuring the morphological parameters of the pelvic floor and for identifying damage to the integrity of the LAM. Although we studied a population that has not been previously evaluated for this method, our results reproduced the existing data in the literature [21, 23].

The intrarater analysis showed that the anteroposterior diameter and hiatal area were the most reliable morphological parameters, and the LoA indicated that they can be useful tools for research and clinical purposes. Considering the interrater analysis, the LoA for these same measurements sufficiently support their careful use in research and clinical practice. Additionally, the static measurements of anteroposterior diameter and hiatal area were consistent with those previously described in studies in women without prolapse [25, 26]. This technique could be systematically used for objective pelvic floor evaluation in women with CPP in future studies. Surprisingly, the hiatal transverse diameter, LAM thickness, and LUG measurements were not reliable, even when we used a technique that minimized subjectivity. Some authors have described good reliability for hiatal transverse diameter measurement [27, 28]. However, other studies on the reliability of LAM biometry and avulsion that used 3D endovaginal ultrasonography and different populations reported similar results to ours. Even after excluding cases of LAM avulsion, the reliability was not significantly improved. We believe that some handling-related technical factors might have interfered in those measures, as follows: (1) angulation, which may have not allowed improved lateral resolution; (2) focus, which could be unique to the level of this region by increasing the lateral resolution; (3) image of the adjacent tissue, which can be similar to the evaluated muscle, thus reducing its definition (however, this effect can be reduced by the OmniView-VCI technique); and (4) acquisition of 3D volume images of the muscles through the cen-

Table 2. Intrarater concordance correlation coefficient and limits of agreement.

	Observer A (time 1)	Observer A (time 2)	CCC (95% CI)	LoA (2.5 to 97.5% limits)	% LoA
Hiatal anteroposterior diameter (mm)	51.5 ± 5.9	51.4 ± 6.2	0.98 (0.97 to 0.99)	−2.2 to 2.4	4.5
Hiatal transverse diameter (mm) *	40.1 ± 3.6	40.6 ± 3.6	0.85 (0.75 to 0.91)	−4.4 to 3.3	9.6
Hiatal area Render (cm^2)	15.0 ± 2.6	15.0 ± 2.7	0.99 (0.98 to 0.99)	−0.8 to 0.8	5.4
Hiatal area Omni- VCI (cm^2)	15.0 ± 2.7	15.0 ± 2.7	0.99 (0.99 to 1.00)	−0.6 to 0.6	3.8
Levator ani thickness 3 h (mm) †	8.6 ± 1.7	7.7 ± 1.1	0.58 (0.42 to 0.70)	−1.4 to 3.2	28.2
Levator ani thickness 9 h (mm) ‡	8.7 ± 1.8	7.8 ± 1.0	0.53 (0.38 to 0.66)	−1.5 to 3.4	29.7
Levator-urethra gap right (mm)	21.9 ± 4.4	22.3 ± 4.0	0.96 (0.94 to 0.98)	−2.6 to 1.6	9.6
Levator-urethra gap left (mm)	21.2 ± 2.7	21.6 ± 2.4	0.89 (0.82 to 0.94)	−2.6 to 2.0	10.7
Urethra-anus distance (mm)	27.1 ± 5.1	26.8 ± 5.0	0.96 (0.93 to 0.98)	−2.3 to 3.1	10.0

Notes: CCC, concordance correlation coefficient; LoA, limits of agreement.

* Hiatal transverse diameter measurement excluding levator injury cases: CCC = 0.83 (0.72 to 0.90); LoA = −4.6 to 3.5; % LoA = 9.6%.

† Levator ani thickness (3 h) measurement excluding levator injury cases: CCC = 0.61 (0.44 to 0.73); LoA = −1.4 to 3.0; % LoA = 28.2%.

‡ Levator ani thickness (9 h) measurement excluding levator injury cases: CCC = 0.57 (0.41 to 0.69); LoA = −1.6 to 3.2; % LoA = 30.0%.

Table 3. Interrater concordance correlation coefficient and limits of agreement.

	Observer A (time 1)	Observer B	CCC (95% CI)	LoA (2.5 to 97.5% limits)	% LoA
Hiatal anteroposterior diameter (mm)	51.5 ± 5.9	52.2 ± 6.2	0.96 (0.94 to 0.98)	−3.5 to 2.1	5.4
Hiatal transverse diameter (mm) *	40.1 ± 3.6	39.5 ± 4.7	0.62 (0.43 to 0.76)	−6.7 to 7.8	18.2
Hiatal area Render (cm^2)	15.0 ± 2.6	15.3 ± 3.0	0.93 (0.89 to 0.96)	−2.2 to 1.7	13.0
Hiatal area Omni- VCI (cm^2)	15.0 ± 2.7	15.2 ± 2.8	0.97 (0.94 to 0.98)	−1.6 to 1.1	8.9
Levator ani thickness 3 h (mm) †	8.6 ± 1.7	7.5 ± 1.0	0.30 (0.12 to 0.46)	−2.0 to 4.1	38.2
Levator ani thickness 9 h (mm) ‡	8.7 ± 1.8	7.5 ± 0.8	0.32 (0.18 to 0.45)	−1.6 to 4.1	35.1
Levator-urethra gap right (mm)§	21.9 ± 4.4	21.5 ± 4.3	0.94 (0.89 to 0.96)	−2.7 to 3.4	14.1
Levator-urethra gap left (mm)	21.2 ± 2.7	21.5 ± 2.6	0.78 (0.65 to 0.87)	−3.7 to 3.2	16.3
Urethra-anus distance (mm)	27.1 ± 5.1	28.2 ± 4.4	0.90 (0.84 to 0.94)	−4.8 to 2.7	13.5

Notes: CCC, concordance correlation coefficient; LoA, limits of agreement.

* Hiatal transverse diameter measurement excluding levator injury cases: CCC = 0.67 (0.47 to 0.80); LoA = −4.6 to 6.5; % LoA = 18.2%.

† Levator ani thickness (3 h) measurement excluding levator injury cases: CCC = 0.45 (0.29 to 0.58); LoA = −1.7 to 3.3; % LoA = 38.2%.

‡ Levator ani thickness (9 h) measurement excluding levator injury cases: CCC = 0.39 (0.25 to 0.51); LoA = −1.6 to 3.7; % LoA = 35.1%.

§ Levator-urethra gap measurement excluding levator injury cases: CCC = 0.82 (0.70 to 0.90); LoA = −2.5 to 1.6; % LoA = 10.7%.

tral or principal lobe of the ultrasonic beam, together with the specific training of the examiner. Another option would be to use the 2D matrix probe, a new technology in 3D/4D ultrasonography, with better resolution and superior image quality.

The endovaginal probe is not a standardized tool for evaluating the pelvic floor. However, its use has several advantages. It has a high frequency and better image resolution, allowing the identification of the normal anatomy and possible existing lesions. Another relevant aspect is that because it is routinely used for pelvic examinations, there is no need to change the transducer, which translates to reduced procedure time, lower cost for sterilization, and greater comfort for patients owing to the smaller contact area, particularly for those with CPP and dyspareunia. The depth of the probe used in this work can reach 16 cm, and the studied area of the pelvic floor ranged from 2 to 4 cm. Therefore, the use of a probe is suitable for evaluating this parameter. Consequently, we believe that the transperineal method is better and should be selected for this purpose, although it may be less effective than the endovaginal method for LUG measurement [29].

The absence of dynamic evaluation of the pelvic floor with the Valsalva maneuver and of the maximal strength of PFMs, thus precluding any functional inference in this population, was the limitation of this study. However, we believe that this does not affect our objectives. The assessments of reliability and agreement are affected by various sources of variability in the measurement setting. Repeated measurements using the same stored images or datasets instead of performing a completely new examination has other unexpected flaws, such as their unsuitability for use in quantifying the perineal transducer compression and in performing a correct Valsalva maneuver.

Transperineal 3D endovaginal probe ultrasound is reliable for identifying relevant injury to the LAM and for measuring morphological parameters, specifically anteroposterior diameter and hiatal area of the pelvic floor in the minimal dimension plane. More studies are needed before measurements of the hiatal transverse diameter, LAM thickness, and LUG can be recommended as objective parameters for pelvic floor evaluation in women with CPP.

Author contributions

OBP, JCRS and AAN designed the research study. MAV performed the research. OBP, MAV and HH analyzed the data. HH, OBP and FSC contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The ethics committee of the hospital approved the study (protocol no. 128,942). All women provided written informed consent before inclusion in the study.

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Conflict of interest

The authors declare no conflict of interest.

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