ORIGINAL ARTICLE



Dynamic translabial ultrasound versus echodefecography combined with the endovaginal approach to assess pelvic floor dysfunctions: How effective are these techniques?

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Abstract

Background The aim of this study was to evaluate the role of dynamic translabial ultrasound (TLUS) in the assessment of pelvic floor dysfunction and compare the results with echodefecography (EDF) combined with the endovaginal approach.

Methods Consecutive female patients with pelvic floor dysfunction were eligible. Each patient was assessed with EDF combined with the endovaginal approach and TLUS. The diagnostic accuracy of the TLUS was evaluated using the results of EDF as the standard for comparison.

Results A total of 42 women were included. Four sphincter defects were identified with both techniques, and EDF clearly showed if the defect was partial or total and additionally identified the pubovisceral muscle defect. There was substantial concordance regarding normal relaxation and anismus. Perfect concordance was found with rectocele and cystocele. The rectocele depth was measured with

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TLUS and quantified according to the EDF classification. Fair concordance was found for intussusception. There was no correlation between the displacement of the puborectal muscle at maximum straining on EDF with the displacement of the anorectal junction (ARJ), compared at rest with maximal straining on TLUS to determine perineal descent (PD). The mean ARJ displacement was similar in patients with normal and those with excessive PD on TLUS.

Conclusions Both modalities can be used as a method to assess pelvic floor dysfunction. The EDF using 3D anorectal and endovaginal approaches showed advantages in identification of the anal sphincters and pubodefects (partial or total). There was good correlation between the two techniques, and a TLUS rectocele classification based on size that corresponds to the established classification using EDF was established.

Keywords Pelvic floor · Ultrasound · Constipation · Rectocele · Cystocele

Introduction

Pelvic floor dysfunction can have multiple components and include symptoms related to urinary and/or fecal incontinence (FI), pelvic organ prolapse and obstructed defecation with different clinical presentations. A complete clinical investigation to quantify the severity of the symptoms, detailed examination of the anal canal and perineal body and quantification of pelvic organ prolapse to determine the grade of the prolapse are required. A series of studies have shown similar results comparing dynamic diagnostic modalities including defecography, dynamic ultrasound through different approaches and/or dynamic magnetic resonance imaging to identify pelvic floor dysfunctions [1-8].

In 2004, Beer-Gabel et al. [4] reported using dynamic transperineal ultrasound to evaluate pelvic floor dysfunctions and compared it with defecography, demonstrating a high degree of concordance, and a series of studies followed their lead [9–12]. The other dynamic ultrasound options, such as dynamic three-dimensional (3D) anorectal ultrasound [Echodefecography (EDF)] [5] combined with the endovaginal approach [13, 14], have the advantage of making it easier to identify anatomical and dynamic pelvic floor abnormalities.

The aim of this study was to evaluate the role of dynamic translabial ultrasound (TLUS) for the assessment of pelvic floor dysfunction, comparing the results with EDF combined with the endovaginal approach (dynamic 3D anorectal and endovaginal ultrasound). The values to determine the grade of rectocele using TLUS were based on EDF.

Materials and methods

Patients

In the period from October 2015 to September 2016, consecutive female patients were invited to participate in the study if they had pelvic floor dysfunction including obstructed defecation symptoms (excessive straining, vaginal splinting or sensation of incomplete evacuation) despite having increased intake of dietary fiber (up to 30 g/day for 3 months), and a Cleveland Clinic Florida (CCF) constipation score >6 [15], with fecal and urinary incontinence (UI). The patients were assessed for FI, defined as the uncontrolled passage of feces or gas for at least 1 month in an individual of at least 4 years of age who had previously achieved control [16], and UI, defined as any involuntary leakage of urine with effort, exertion, sneezing, or coughing, and/or leaking or loss of urine associated with an urge to urinate [17].

Patients were excluded if they had organic pathology of the colon or rectum detected by clinical examination or colonoscopy.

The clinical protocol was approved by the Research Ethics Committee of the Walter Cantido University Hospital, and all patients gave written informed consent.

Procedures

Each patient underwent EDF combined with the endovaginal approach and TLUS to evaluate pelvic floor dysfunction. The examinations were performed by colorectal surgeons with experience in evaluating pelvic floor anatomy and dysfunction using dynamic imaging methods: EDF and endovaginal assessment were performed by A.S.V. and TLUS by S.M.M.R., with the examiners being blinded to the results of each technique and the patient's clinical. The results were compared.

Echodefecography combined with the endovaginal approach

The scan was performed with a 3D ultrasound device (Pro-Focus, endoprobe model 2052, B-K Medical, Herlev, Denmark) placed in the rectum, with proximal-to-distal 6.0 cm automatic scans. Patients received a rectal enema and were examined in the left lateral position. Images were acquired by four automatic scans [5, 18].

Scan I (at rest) verified the anatomical integrity of the anal sphincters. Defects were considered to be partial (partially compromised length of the muscle) or total (whole length of the muscle compromised) (Fig. 1).

Scan 2 evaluated the movement of the puborectalis muscle and the external anal sphincter during straining, identifying normal relaxation, non-relaxation or paradoxical contraction (anismus).

Scan 3 quantified the perineal descent (puborectalis muscle (PR) descent) by measuring the distance between the position of the proximal border of the PR muscle at rest and the point to which it was displaced by maximum



Fig. 1 Three-dimensional anorectal ultrasound image of anal sphincter intact. *EAS* external anal sphincter, *IAS* internal anal sphincter, *PR* puborectalis muscle

straining. Perineal descent ≤ 2.5 cm was classified as normal, while PD >2.5 cm was classified as excessive.

For scan 4, 60–120 ml ultrasound gel was injected into the rectal ampulla. This scan identified and quantified the depth of rectocele as well as intussusception, sigmoidocele/ enterocele (grade II or III) and cystocele (measured by a displacement of the bladder or bladder neck below the proximal margin of the PR \geq 0.5 cm). Rectocele depth was measured by drawing two parallel horizontal lines adjacent to the posterior vaginal wall, one in the initial straining position and one at the point of maximal straining. The distance between the two vaginal wall positions was used to determine the rectocele depth. Rectocele depth was classified as grade I (<0.6 cm mm), grade II (0.6–1.3 cm) or grade III (>1.3 cm).

All patients who had a vaginal delivery underwent 3D endovaginal ultrasound to evaluate the anatomical integrity of the pubovisceral muscles (PVM) (including the puborectalis and pubococcygeus muscles) (Fig. 2) or defects [13, 14]. Patients were placed in the dorsal lithotomy position. The same transducer, using 12 MHz and a focal distance of 5.2 cm, was placed in the vagina in the neutral position, and the endoprobe was introduced as far as the bladder. Pubovisceral defects were defined as complete or partial detachment (discontinuity) of the muscle from the pubic rami.



Fig. 2 Three-dimensional endovaginal ultrasound image of pubovisceral muscles intact (PMV)

Translabial ultrasound

TLUS was performed with conventional convex transducers, with frequencies of 6 MHz and field of view at least 70° (B-K Medical Type 8802, ProFocus Peabody, Massachussets USA), placed on the perineum, which provided two-dimensional imaging of the pelvic floor. Patients received a rectal enema and were examined in the dorsal lithotomy position, with hips flexed and abducted. Imaging is usually performed with the patient at rest and during maximal Valsalva maneuver [4, 9].

In the mid-sagittal plane, all anatomical structures can be seen from posterior to anterior (bladder, urethra, vagina, anal canal and rectum) between the posterior surface of the pubic symphysis and the posterior part of the levator ani (Fig. 3).

Anismus was characterized by a failure to execute the relaxation of the puborectalis muscle and the external anal sphincter that is required for successful defecation. It was measured by the anorectal angle (the confluence of lines through the hypoechoic band representing the posterior internal anal sphincter and through the posterior wall of the rectal ampulla) at rest and during evacuation. Thus, anismus was recorded when the anorectal angle failed to open or became narrower during straining compared to the anorectal angle at rest. In cases with normal relaxation, the angle increases.

The rectum was filled with 60 mL of acoustic gel to identify rectocele and quantify rectocele depth. The rectocele depth was measured perpendicular to a line



Fig. 3 Translabial ultrasound image of pelvic floor. PS pubic symphysis, U urethra, V vagina, EAS external anal sphincter, IAS internal anal sphincter, PR puborectalis muscle

projected along the contour of the anterior rectal wall. The depth of rectocele using the TLUS technique was established measuring the rectocele sizes in the same patients and comparing with EDF results.

Rectal intussusception was characterized as an invagination of the rectal wall into the rectal lumen during maximal Valsalva maneuver. Entero-sigmoiodocele was diagnosed when there was the presence of bowel loops into the pelvis between the rectum and vagina. Cystocele was identified using a reference line drawn parallel to the inferoposterior margin of the pubic symphysis and the downward displacement of the bladder beyond the inferoposterior margin of the pubic symphysis during the Valsalva maneuver.

Perineal descent was measured by the displacement of the anorectal junction (ARJ) in relation to pubic symphysis, calculating the difference of the vertical line between the ARJ and a reference line drawn parallel to the inferoposterior margin of pubic symphysis, comparing the distance between at rest and maximal straining position.

Statistical analysis

Differences between anorectal angle and anorectal position were assessed using Student's *t* test. The Spearman rank correlation coefficient (ρ) was calculated to evaluate the relationship between the displacement of the PR muscles upon maximum straining on EDF, with the difference of the distance between the anorectal junction and a line from the pubic symphysis at rest compared to maximal straining on TLUS. The diagnostic accuracy of TLUS was evaluated considering the results of echodefecography as the standard for comparison. As an index of concordance between the methods, *kappa* (κ) coefficients were calculated with 95% CI [19]. Concordance based on the κ value was classified as slight (0–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), near perfect (0.81–0.99) or perfect (1.0) [19]. Results were considered statistically significant when p < 0.05.

Results

A total of 42 women were included in the study, with a mean age of 56 years (range 26–85). All of them complained of ODS. The median CCF constipation score 9 (range 7–21), 16 (38%) complained of FI and 17 (40%) of UI. Overall, 29 (69%) had vaginal deliveries, 6 had undergone cesarean sections and 7 were nulliparous.

Echodefecography

A total of seven (17%) had sphincter and/or PVM defects: Two had partial external anal sphincter (EAS), two combined partial EAS plus internal anal sphincter (IAS) associated with unilateral PVM muscles defects and three had PVM defects with intact sphincters. Five of these patients complained of FI symptoms.

The anorectal angle increased from a mean of $87^{\circ} \pm 5.5^{\circ}-98^{\circ} \pm 12.2^{\circ}$ (mean difference, $+11^{\circ}/p = 0.00$) in 22 (52%) patients, and failed to open in 20 (48%) patients, decreasing from a mean of $86^{\circ} \pm 4.6^{\circ}$ to $80^{\circ} \pm 5.7^{\circ}$, with a mean difference of -6° (p = 0.00).

Rectocele was identified in 27 (64%) patients and classified as grade I in 4 patients, grade II in 5 and grade III in 18, and as a significant rectocele (grade II or III) in a total of 23 (55%) patients. Rectal intussusception was found in 17 (40%) patients. Eleven of the patients with intussusception also had significant rectocele. An enterocele-sigmoidocele was observed in one patient. Perineal descent was identified in 10 (24%) patients, and the mean displacement of PR muscles was 2.9 cm (range 2.6–3.5 cm). Cystocele was identified in 20 (48%) patients, 10 of whom complained of urinary incontinence.

Translabial ultrasound (TLUS)

TLUS identified sphincter defects in four (10%) patients without demonstrating if the defect was a partial (partially compromised length of the muscle) or total (whole length of the muscle compromised). Two of these patients had EAS defects and two EAS plus IAS. Three complained of FI symptoms. No PVM defects were identified.

The anorectal angle increased from a mean of $106^{\circ} \pm 29.6^{\circ}-148^{\circ} \pm 30.7^{\circ}$ (mean difference, $\pm 12^{\circ}$) in 19 (45%) patients, and failed to open in 23 (55%) patients, decreasing from a mean of $110^{\circ} \pm 25^{\circ}$ to $98^{\circ} \pm 21^{\circ}$, with a mean difference of -6° (p = 0.043).

Rectocele was identified in 25 patients. Different depths of rectocele were measured at TLUS and quantified according to the EDF classification: The depth was >2.0 cm for grade III, from 1.1 to 2.0 cm for grade II and ≤ 1.0 cm for grade I. A total of 17 patients were classified with grade III; 6 with grade II and 2 with grade I.

Rectal intussusception was found in three (7%) patients, all of whom had rectocele.

An enterocele-sigmoidocele was observed in one patient. The mean ARJ position at rest was 2.2 ± 0.8 cm (range 0.0–4.9 cm) and 1.1 ± 1.0 cm (range -1.0 to 2.8/p = 0.00)

during maximal straining. The mean displacement of the anorectal junction was 1.1 ± 0.87 cm (range 0.0–2.9).

Cystocele was identified in 19 (45%) patients.

Concordance between EDF and TLUS

The both modalities identified the sphincter muscles defects, but TLUS did not determine the length of the sphincter and PVM defects.

Table 1Concordance betweenechodefecography (EDF) andtranslabial ultrasound (TLUS)regarding normal relaxation andanismus diagnosis

	Translabial ultrasound	Total diagnosed by ED		
	Normal relaxation	Anismus		
Echodefecography				
Normal relaxation	17*	5	22	
Anismus	2	18*	20	
Total diagnosed by TLUS	19	23	44	

* Concordant findings



Fig. 4 Comparison of the anorectal angle at rest and during straining in patients with anismus assessed with echodefecography. *EAS* external anal sphincter, *IAS* internal anal sphincter, *PR* puborectalis

Anismus was identified in 18 (43%) patients and normal relaxation in 17 (40%) with both techniques. Substantial concordance was observed between EDF and TLUS ($\kappa = 0.68, 95\%$ CI 0.37–0.97) (Table 1; Figs. 4, 5).

Rectocele was identified in 27 (64%) and 25 (59%) patients by EDF and TLUS, respectively, with perfect concordance ($\kappa = 0.98, 95\%$ CI 0.69–1.0). The two techniques demonstrated identical findings in 15 patients without rectocele, and in 2, 5, and 17 with grade I, II, and III, respectively (Fig. 6). Different rectocele depths were measured with TLUS and quantified according to the EDF classification, i.e., >2.0 cm for grade III, from 1.1 to 2.0 cm for grade II and ≤ 1.0 cm for grade I. Two cases of grade I identified by EDF were not identified on TLUS (Table 2).

Intussusception was identified in 17 (40%) patients on EDF and confirmed in 3(7%) by TLUS with fair concordance ($\kappa = 0.20, 95\%$ CI 0.02–0.39) (Table 3; Fig. 7). Entero-sigmoicele was identified in one (2%) patient by both techniques.

muscles. **a** Angle measured at rest (*white lines*). **b** Decreased angle during straining (*white lines*)

Cystocele was identified in 20 patients by EDF and confirmed in 18 by TLUS with perfect concordance ($\kappa = 0.85, 95\%$ CI 0.55–1.0) (Table 4; Fig. 8).

On EDF, excessive PD, and PR muscle displacement >2.5 cm was detected in 10 patients and the mean ARJ displacement was 1.2 ± 0.86 cm (range 0.1-2.9 cm), using TLUS 32 patients had normal PD, and PR muscle displacement ≤ 2.5 cm, on EDF and the mean ARJ displacement was 1.1 ± 0.86 cm (range 0.0-2.9), using TLUS. The displacement of the ARJ on TLUS was similar when comparing patients with normal PD and excessive PD. (Table 5; Fig. 9).

There was no positive correlation between the displacement of PR muscles at maximum straining on EDF (mean = 2.2 ± 0.58 cm, range 0.8–3.5) with the displacement of the ARJ (mean = 1.1 ± 0.85 cm, range 0.0–2.9), at rest versus at maximal straining on TLUS (r = -0.03; p = 0.86).



Fig. 5 Comparison of anorectal angle at rest and during straining in patients with anismus as assessed with translabial ultrasound. *EAS* external anal sphincter, *IAS* internal anal sphincter, *PR* puborectal

muscles, AC anal canal, V vagina. **a** Angle measured at rest (*white lines*). **b** Decreased angle during straining (*white lines*)



Fig. 6 Detection of rectocele (grade classified). **a** Echodefecography—Line 1 = measures the depth of the rectocele. **b** Translabial ultrasound—Line 2 = measures the depth of the rectocele (**b**). *PS*

pubic symphysis, U urethra, V vagina, AC anal canal, EAS external anal sphincter, IAS internal anal sphincter, PR puborectalis muscle

Discussion

The present study showed that TLUS has a good correlation with EDF for the diagnoses of anismus, rectocele and cystocele. We chose EDF combined with the endovaginal approach as the standard for comparison with TLUS because it has been standardized [5] in comparison with defecography, and validated in a multicenter study [20]. Previous studies have demonstrated the anatomy of the PVM and identified defects in patients who had a vaginal delivery, using 3D endovaginal ultrasound, as well as positive correlation between sphincter and/or PVM defect and the severity of the CCF incontinence score in females with FI [14]. Therefore, the techniques (EDF with

Table 2	Concordance	between	echodefecography	(EDF)	and translabial	ultrasound	(TLUS)	regarding	rectocele and	grade
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	Translabial ultrasound				Diagnosed by EDF
	Without rectocele	Rectocele I	Rectocele II	Rectocele III	
Echodefecography					
Without rectocele	15*	0	0	0	15
Rectocele I	2	2*	0	0	04
Rectocele II	0	0	5*	0	05
Rectocele III	0	0	1	17*	18
Diagnosed by TLUS	17	02	06	17	42

* Concordant findings

Table 3Concordance betweenechodefecography (EDF) andtranslabial ultrasound (TLUS)regarding diagnosis ofintussusception

	Translabial ult	Diagnosed by ED	
	Without	Intussusception	
Echodefecography			
Without	25*	0	25
Intussusception	14	3*	17
Diagnosed by TLUS	39	03	42

* Concordant findings



Fig. 7 Detection of intussusception (arrows) with echodefecography (a) versus translabial ultrasound (b)

endovaginal) were combined using the same transducer, making a complete evaluation possible. A total of 29 (60%) of the patients of this study had a vaginal delivery and 7 (24%) had isolated or combined sphincter defects and/or PVM defects. All patients who had a vaginal delivery should be evaluated with both modalities, even without FI symptoms, to identify occult lesions [21–24].

Overall, multiple pelvic floor disorders were prevalent in this group of patients, because the study population included females with multiple symptoms, such as ODS associated with UI and/or FI in approximately 40%, similar to a series of published studies [25–27].

All the patients in the present study received a rectal enema 2 h before the examination in order to avoid

	Translabia	al Ultrasound	Diagnosed by EDI	
	Without	Cystocele		
Echodefecography				
Without	21*	1	22	
Cystocele	2	18*	20	
Diagnosed by TLUS	23	19	42	

 Table 4 Concordance between both techniques regarding cystocele

* Concordant findings

artefacts, such as feces and air. As in the original study described by Beer-Gabel et al. [3], the rectum was filled with ultrasonographic coupling gel (60 ml) to visualize the anatomical structures and cause the patients to feel the stimulus to evacuate.

The values of the angles at rest and during straining were not compared between the techniques because the landmarks used to draw the angles were not similar. However, the results in patients identified as having normal relaxation or anismus showed substantial concordance. Grasso et al. [19] in contrast, Perniola et al. [12] also compared TLUS with defecation proctography and found poor concordance in the measurements of the anorectal angle. The present study revealed high concordance rates between EDF and TLUS for detecting rectocele, with only two cases of grade I rectocele not detected on TLUS. Previous studies using TLUS have quantified rectocele from the herniation of at least 10 mm in depth of the rectal contents into the vagina [4]. Perniola et al. [12] comparing TLUS and proctography considered defecation proctography to be the gold standard and used the same values to measure rectocele depth, which showed very poor concordance. Therefore, in the present study the cutoff values for each grade of rectocele using TLUS were determined based on EDF instead of using the same measurements previously determined by defecography, considering that the techniques, markers and positions of the patients were different.

A total of 17 cases of intussusception were found using EDF, with the majority of these associated with significant rectocele, however, only three cases were detected on TLUS, revealing fair concordance. Even with gel inserted into the rectum to distend it and make it possible to visualize the layers of the rectal wall, TLUS was unable to identify the majority of intussusception cases. These results are in accordance with those reported in the literature [9, 12].



Fig. 8 Detection of cystocele. a Echodefecography—Line 1 = line dawn in the proximal margin of the puborectalis muscles (PR)/Line $2 = \text{measures the displacement of the bladder or bladder neck below the proximal margin of the PR. b Translabial ultrasound—Line <math>1 = \text{line}$ drawn parallel to the inferoposterior margin of the pubic

symphysis/Line 2 = measures the displacement of the bladder beyond the inferoposterior margin of the pubic symphysis during the Valsalva maneuver. *PS* pubic symphysis, *EAS* external anal sphincter, *IAS* internal anal sphincter, *PR* puborectalis muscles

Table 5 Perineal descent measurements: echodefecography (EDF) compared with translabial ultrasound (TLUS)

Perineal descent measurements	Normal perineal descent $N = 32$ patients (cm)	Excessive perineal descent $N = 10$ patients (cm)	р
Puborectalis muscle displacement on EDF mean (SD)	1.9 (0.39)	3.0 (0.28)	0.00
Anorectal junction displacement on TLUS mean (SD)	1.1 (±0.86)	1.2 (±0.86)	0.89



Fig. 9 Perineal descent measurements at rest (a) and during the Valsalva maneuver (b). Line 1 = Line drawn parallel to the inferoposterior margin of pubic symphysis/Line 2 = Vertical line from the anorectal junction to the *line* drawn parallel to the inferoposterior margin of pubic symphysis comparing the at rest

(a) with during the Valsalva maneuver (b). Line 3 = The anorectal angle at rest (a) and during the Valsalva maneuver (b). *EAS* external anal sphincter, *IAS* internal anal sphincter, *PR* puborectalis muscles, *PS* pubic symphysis

Only 1 case of enterocele was detected in this study, diagnosed by both techniques. Few studies have compared transperineal ultrasound with dynamic evacuation proctography and the majority of studies do not mention the diagnosis of enterocele [10, 12].

The technique of assessment of cystocele was described using a reference line drawn parallel to the inferoposterior margin of the pubic symphysis and the downward displacement of the bladder beyond the inferoposterior margin of the pubic symphysis during the Valsalva maneuver. Cystocele was also considered if the bladder was descending and in ascertaining the configuration of the bladder neck and urethra [28, 29]. Lone et al. [30] evaluated the displacement of pelvic organs using this reference line and compared this with the validated Pelvic Organ Prolapse Quantification System (POP-Q), showing that the proportion of correct predictions was 60% for bladder displacement. The present study evaluated bladder displacement using TLUS comparing this with EDF that measured the displacement of the bladder or bladder neck ≥ 0.5 cm below the proximal margin of the PR. Thus, even when a different technique and anatomical reference points were used the results demonstrated good concordance. In those patients with symptoms of UI, cystocele should be evaluated with complementary methods in order to choose the best modalities of treatment.

Beer-Gabel et al. [4] described the original technique for determining perineal descent comparing with defecography. The results were similar concerning the position of the ARJ at rest and during straining. The present study demonstrated that there was a significant displacement in the ARJ position when comparing at rest with maximal straining. On the other hand, the displacement of the ARJ by TLUS was similar when comparing patients with normal PD and with excessive PD and there was no positive correlation between the displacement of PR muscles at maximum straining by EDF with the displacement of the ARJ by TLUS. In the 10 patients identified on EDF as having excessive PD, the difference between the ARJ position in relation to then line from pubic symphysis when comparing at rest to maximal straining was not enough to quantity as an excessive PD by TLUS.

A series of studies have demonstrated that the advantages and disadvantages of the modalities to evaluate the pelvic floor dysfunction. Vitton et al. [8] compared dynamic ultrasound with dynamic magnetic resonance imaging using defecography as a gold standard. They obtained similar results regarding pelvic floor dysfunction and found that patient tolerance was significantly better for dynamic anorectal endosonography (72%) than for dynamic resonance (25%) or defecography (2%). The other advantage to using ultrasound is cost-effectiveness, as it can be performed in the doctor's consulting room.

Further studies should compare TLUS with dynamic magnetic resonance imaging to demonstrate the correlation between the techniques. A multicenter study should also be performed to evaluate the results of different examiners and the inter-observer reliability.

Conclusions

Dynamic ultrasound is a good option for evaluating patients with pelvic floor dysfunction and can be considered first line depending on the symptoms of the patients and the availability of the ultrasound examination. There is a good correlation when EDF and TLUS and both techniques can be used to evaluate pelvic dysfunction. However, EDF is more effective in evaluating the muscle integrity and identifying defects.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The clinical protocol was approved by the Research Ethics Committee of the Walter Cantido University Hospital.

Informed consent All patients gave written informed consent.

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