Usefulness of real time elastography strain ratio in the assessment of cervical intraepithelial neoplasia and cervical cancer using a reference material

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Abstract

Aims: To assess the usefulness of real time elastography (RTE) strain ratio (SR) in diagnosing cervical cancer (CC) and cervical intraepithelial neoplasia (CIN), using a synthetic experimental device (ED) as reference material. **Material and methods:** Seventy-nine participants were enrolled, divided in three groups: Group 1 – benign cervix (n=39); Group 2 – CIN (n=32); Group 3 – CC (n=8). Transvaginal RTE was performed, with SR determination, as the ratio between the ED and the cervical tissue. Mean SR values of the groups were compared; diagnostic performance was assessed by tracing the receiver operating characteristic (ROC) curve. Area under the curve (AUC) was analyzed. Cut-off values were established. Pathological results were considered as reference for data interpretation. **Results:** SR means significantly differed in Group 1 as compared to Groups 2 and 3 (p=0.001). Excluding 2 aberrant values in Group 3, assigned to cases complicated by hemorrhagic necrosis, statistical difference was also noted between Groups 2 and 3 (p=0.02). For Groups 1 and 3, AUC was 0.966 with a 95%CI (0.914-1.000); the cut-off point of SR was 1.42, with a sensitivity of 100% and a specificity of 94.9%. AUC was 0.752 with a 95%CI (0.629-0.876) for Groups 1 and 2. For the cut-off value of 1.03, sensitivity and specificity were 75% and 74%, respectively. **Conclusion:** RTE SR, performed with a synthetic reference material, seems a reliable method for distinguishing between benign uterine cervix and malignancy, with promising results as a complementary investigation in diagnosing CIN. However, SR becomes inoperant in cases of cancer complicated with hemorrhagic necrosis.

Keywords: real time elastography; uterine cervix; cervical intraepithelial neoplasia; cervical cancer; strain ratio

Introduction

Cervical cancer (CC), as the gynecological malignancy with the highest mortality rate worldwide and cervical intraepithelial neoplasia (CIN), as its' precursor, require the improvement of diagnostic methods [1-5].

Real-time elastography (RTE), an imaging technique complementary to conventional ultrasonography, can

demonstrate information about tissue stiffness, which, in its' turn, might change under the influence of inflammatory or tumoral processes [2]. RTE is based on the assessment of tissue dislocation, therefore providing an estimate of tissue strain and its' opposite, tissue stiffness. The information is coded in colors - usually, red is chosen to define structures with a high level of dislocation and low stiffness whereas blue represents structures with low strain and high stiffness. Intermediate structures appear encoded in green, but the choice of red-blue colors can be performed by the operator for most ultrasound machines.

The evaluation of colors by using color scores is a subjective semiquantitative interpretation technique. However, RTE also allows semiquantitative stiffness measurement, by determining the Strain Ratio (SR). This

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represents the ratio of dislocation between two structures on the same image [2].

While RTE of the uterine cervix has been intensively investigated, in recent years, for the premature birth prediction [6-11], there are only a few studies focusing on the potential of this method in diagnosing CC. These studies have used different anatomical structures as stiffness reference for the cervix and have not been able to demonstrate any use of RTE in diagnosing CIN [2,5,12-15].

Due to the proximity, the tissues around the cervix, themselves, undergo structural changes in case of cervical disease. As such, the use of pericervical tissue in situ does not provide a constant and reproducible standard of comparison.

The aim of the current study is to assess the usefulness of RTE SR in diagnosing CC and CIN, using a synthetic reference material.

Material and methods

Patients

The design and protocol of this prospective study were approved by the Ethics Committee of the institution (96/63/08.03.2017). After explaining the nature of the study and of the procedures involved, verbal and written informed consent was obtained from all participants. Between April 2017 and June 2019, 79 patients hospitalized at the 2nd Obstetrics and Gynecology Department were enrolled in the study.

Recruitment process of the patients is described in the diagram presented in figure 1. Inclusion criteria represented patients admitted for either cervical biopsy, based on abnormal cytological results at their screening examination, or for conization or hysterectomy, that would allow having a final pathological result of the cervical tissue. Patients with previous cancer treatment (including chemotherapy and radiotherapy on the pelvic area), prior conization, cervical amputation or hysterectomy, as well as clinically visible cervical scars, genital prolapse, marked vaginal atrophy, vaginal stenosis or prior colpocleisis were excluded from the study. The patients presenting important vaginal bleeding or important pelvic pain were not considered for RTE examination.

Subjects were divided in 3 groups, based on the pathological results: Group 1 – control group, where no CIN or CC was detected; Group 2 – CIN and Group 3 – CC.

Reference material design

For the purpose of this study, we conceived an experimental device (ED) consisting of a rounded tip silicone cylinder with a diameter of 2 cm and a length of 3 cm. This device was virtually designed using MeshMixer

Fig 1. STARD diagram of the recruitment process of the patients.

(Autodesk, USA) CAD software and produced out of a grey photopolymer resin through additive manufacturing via stereolithography by using a Form 2 (Formlabs, USA) 3D printer. After manufacturing, we obtained a negative mould of this device by using Elite Double 32 (Zhermack, Italy) duplication silicone. The inside of the mould was then sprayed with a release agent and ZA 13 (Zhermack, Italy) poly addition silicone rubber, with a mixing ratio of 1 part of base to 1.6 parts of catalyst, was poured into the mould in order to obtain the actual device to be used for patient examination. After curing at room temperature for 24 hours, the device was removed from the mould. For RTE assessment, 80 identical single use devices were created (fig 2).

Fig 2. Silicone experimental device used as reference material.

The ED consists of a custom-made material, based on a standard silicone widely used in dentistry. The material was obtained by a standardized variation of the ratio between the base and the catalyst, as described above. Since the preparation recipe is standard, the resulting compound always has the same characteristics. This type of material was chosen due to its similar consistency with the cervix, as well as the fact that its texture produces relatively uniform echoes, which allow speckle tracking and strain elastography. The experimental validation of the material in comparison with standard silicones is the subject of another research.

RTE – image acquisition and evaluation

All the examinations were performed on an Aplio 300 (Toshiba Medical Systems, Tokyo, Japan) ultrasound machine, equipped with a PVT-781VT 3.0/11.0 MHz end-fire endocavitary probe. The patients were asked to empty their bladder before the examination and lie in a lithotomy position. The ED, covered in a thin layer of ultrasound gel, was inserted in a sterile condom, which was knotted, in order to avoid air penetration. A thin thread of 15 cm length was attached to the end of the condom, in order to allow external manipulation of the ED. The ED was then introduced in the posterior fornix of the vagina, right below the uterine cervix. The transducer, covered in a disposable sheath to prevent cross infection, was placed into the vagina, in contact with both the cervix and the ED. Conventional B-mode ultrasound was performed prior to RTE; a sagittal view of the cervix was obtained, with the location of the ED. The external thread was gently manipulated in cases with difficulty in locating the ED. The system was then switched to the elastographic mode. The image was focused on the uterine cervix and the adjacent ED, occupying 2/3 of the screen. The size of the elastography region of interest (ROI) sector was set as follows: the width of the sector was set to maximum, the depth of the sector was set between 4 and 6 cm, in order to encompass as much reference tissue as possible, still preserving the requirement that the area of the cervix and ED should occupy 2/3 of the image. Efforts were made to place the cervix and the ED in the center of the image, to avoid lateral stiffness artefact. The parameters were set as follows: Frame Rate, 8; ApliPure, 5; Precision, 5; Frequency, 7; Focus, 24%; Scan Range, 53%; Dynamic Range, 55 dB; Gamma, 10; Focal Type, 1; Automated Gain Control, 1; Edge Enhancement, 1; Persistence, 2; Guide Size, M; Map, 2.

A light displacement force was applied with the transducer, in the form of light repetitive compressions. The device's software includes an exam quality indicator box; to consider the displacement force as optimal this box must be colored in green.

Fig 3. Selection of ROIs on the RTE image: first ROI (T1) is placed on the cervix, second ROI (R) is traced on the ED; automatic computing of the SR.

A simultaneous view of a gray scale image on the right side of the screen and the elastographic image, with the color-coded stiffness map on the left side of the screen was obtained. The allotment of the colors was chosen as follows: red - low stiffness, blue - increased rigidity and green, intermediate stiffness. After obtaining an adequate image, two circular ROIs were traced on the grey scale image, the first on the cervix, which was the tissue to be analyzed (T1) and the second on the ED, which served as a reference material (R) (fig 3).

In choosing the site for ROI placement (cervix and ED) we always avoided the soft rim artefact at the margin of the cervix and we placed the ROI in the area with the most constant hue over repeated measurements, keeping both ROIs at the same depth as much as possible

SR was automatically computed by the device's software, as R/T1. For each patient, 10 adequate images were obtained, on which SR was determined, the final value of SR being the mean of 10 consecutive measurements.

All procedures were performed by a single examiner (MDS), with 5 years of experience in elastography.

Pathological examinations

Cervical samples, obtained through biopsy, conization or hysterectomy, were fixed, embedded, stained and assessed by pathologists according to standard techniques. All surgical procedures were performed within 36 hours after RTE. All patients with CIN and cancer underwent either conization or hysterectomy. The pathologic procedure was carried out after elastography, therefore no attempt was made to match the area of the ROI with pathology, since the pathologic diagnosis was established by examining the whole specimen. Pathological results served as reference for all data interpretation.

Statistical analysis

Data analysis was performed with SPSS version 24 for Windows (IBM Corporation, NY).

Variance analysis was assessed with the Fisher's test. Student's T, Independent Samples Kruskal-Wallis Test and Mann-Whitney U Test were performed to evaluate the age and mean SR difference between the three groups. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic performance of the method. Area under the curve (AUC) was analyzed and best cut-off values were established, in accordance with sensitivity and specificity.

Clinical information of the subjects was available to the performer of the ultrasound examination. Pathology results were available *post hoc*, therefore the performer was blinded to pathology.

There were no indeterminate tests or missing data. Acquisition of data was continued until the number of participants was greater than 30 in at least 2 sample groups.

Results

A total of 79 participants (mean age 42.48 years) were enrolled. Of these, 39 patients had benign cervical findings; 32 were diagnosed with CIN [10 cases with CIN I (31.25%), 6 with CIN 2 (18.75%) and 16 with CIN 3 (50%)]; and in 8 patients CC was detected, all of which were squamous cell carcinomas (cancer in situ – 1 case, IA stage – 3 cases, IB stage – 1 cases, IIA stage – 1 case,

Fig 4. SR determination in the study groups: a) benign cervix; b) CIN; c) CC.

Fig 5. Mean SR value in the study groups.

IIIA – 1 and stage IIIB – 1 case, FIGO Classification). Mean age in the three study groups was: 45.38 years in Group 1, 38.68 years in Group 2, 43.5 years in Group 3, significantly different only between Groups 1 and 2 ($p=0.001$).

Representative images for each group are illustrated in figure 4.

Mean SR value was, as follows: 0.89 in Group 1, 1.42 in Group 2 and 1.75 in Group 3 (fig 5). However, 2 outlying values were noted in Group 3 (0.71 and 0.85), which had been attributed to patients diagnosed with CC complicated with hemorrhagic necrosis. After excluding the outliers, mean SR was 2.07 for Group 3.

Student's T test revealed significant difference between mean SR of Groups 1 and 2 ($p=0.001$). Comparison of initial data by the Mann-Whitney U test indicated significant difference between mean SR of Groups 1 and 3 ($p=0.001$), but no difference between Groups 2 and 3 ($p=0.21$). After the exclusion of outlying values in Group 3, analysis indicated significant difference between Groups 2 and 3 ($p=0.02$).

Clinical value of the SR was evaluated by ROC curve analysis. When comparing Groups 1 and 3, AUC was 0.966 with a 95% CI (0.914-1.000). The best cut-off point of SR was 1.42, with a sensitivity of 100% and a specificity of 94.9%. For Groups 1 and 2, AUC was 0.752 with a 95%CI (0.629-0.876). For the cut-off value of 1.03, sensitivity and specificity were 75% and 74%. The AUC for groups 2 and 3 was 0.797, 95% CI (0.648-0.946) and the cut-off point was established at 1.51, with a sensitivity of 100% and a specificity of 65%.

There were no adverse effects as a consequence of RTE.

Discussion

RTE implies the application of a displacement force with the transducer, and the stiffness of the structures is assessed according to their level of dislocation. To date, RTE has found clinical applicability in thyroid, breast and prostate pathology [16-18]. The results obtained by this technique can be graded semiquantitatively either subjectively by assigning color-scoring scales after color analysis of the image or by computing the SR between the analyzed tissue and a reference structure. Strain measured in a single ROI, as a relative expression of stiffness, does not provide an absolute value [19].

In 2007, Thomas et al designed the first study attempting to investigate the basic tissue elastic properties in the healthy and pathologic cervix, concluding that elasticity scores allow differentiation of malignancy from normal findings, but cannot identify CIN [14]. Other studies also concluded that RTE scores are a promising technique, but are limited in detecting early CC stages [5,12,15].

In previous studies related to uterine cervical malignancy, SR has been computed: between cervical and parametrial tissue, concluding that RTE was useful in the differential diagnosis of CC and in the assessment of infiltration [2]; between the lowest segment of the uterine corpus and the cervical tumor, suggesting that RTE is an effective method for predicting radiotherapy response [13]; or between cervical lesions and normal cervical tissue, indicating that RTE has a high clinical value in differentiating benign from malignant cervical lesions [12]. Most studies have shown that CC is more rigid than normal cervical tissue [2,5,12]. However, a single study reported that CC had greater strain, thus being significantly softer than normal surrounding tissue [13].

The initial analysis of our data indicated the absence of significant differences between the normal and CC group. Two aberrant values in a small group (n=8) were attributed to patients presenting with advanced cancer, complicated with hemorrhagic necrosis, which explains the decrease in rigidity determined by RTE SR. After excluding the two aberrant values from the statistical analysis, the CC group appeared to have significantly increased rigidity as compared to both the normal and the CIN group. The presence of hemorrhagic necrosis could probably explain the results obtained by Mabuchi et al [13].

The challenge in using SR is the choice of reference tissue to which the cervix is reported. The parameters' stiffness might vary from one patient to another; the uterine body's strain may also differ as a result of the presence of pathological processes (leiomyomas, adenomyosis) or hormonal or age-dependent changes; the ratio of

normal to tumoral cervical tissue might be relative, since tumor margins cannot be certainly delimited on an ultrasonographic image [11,12,14]. Therefore, we tested the usefulness of a synthetic reference ED. The cervix of all patients was compared to a constant rigidity marker and SR data was used to compare the rigidity of the cervix between the three groups.

The only other published study with a synthetic reference material used a cap over the tip of the transducer [20]. A limitation of this technique, described by the authors, was a maximum distance of 15 mm for which the examination was relevant. By interposing the cap between the transducer and the cervix, the two structures to be compared were located at different depths from the transducer. To avoid this limitation, we designed a device placed parallel to the cervix so that the displacement force was applied from the same level on both structures. Unlike the study conducted by Hee et al [20], our ED did not have a known Young modulus. We do not consider this a limitation as we did not intend to calculate the Young modulus of the cervix, but to test SR as a diagnostic tool.

To the best of our knowledge, this is the first study using a synthetic material as a stiffness reference in the assessment of elasticity by RTE SR in CC and CIN.

Our results differ from those of other researchers, who showed that normal uterine cervix cannot be differentiated from CIN, and also CIN from early cancer, by RTE with a biological tissue as a reference. Indeed, it is not expected that currently-available elastographic techniques would be able to detect changes limited to several cellular layers (cervical epithelium). In SR we used a relatively large ROI, which included predominantly cervical stroma. As such, our observations can only be explained by changes in cervical stroma in CIN patients.

A study conducted by Bumrunghai et al has demonstrated that micro-RNA21, recognized as an oncomir in many types of human malignancy, is involved in cervical lesions and its up-regulation in tumor-stroma might be involved in the inflammation process and cervical cancer progression [21]. Another paper has reported that high levels of c-Myc protein, the product of the proto-oncogene c-myc, were found in both malignant and pre-malignant cervical lesions, suggesting that the c-Myc expression in stromal cells might induce a tumor promoting microenvironment [22]. Furthermore, interleukin-6 (IL-6), as a fibroblastic product, is a cytokine implied in cervical cancer pathogenesis, which has often been detected in cervical stroma in cancer patients [21]. Other chemokines, cytokines and growth factors might be involved due to abnormal fibroblast function. It has also been reported that VEGF (vascular endothelial growth

factor) and FGF2 (basic fibroblastic growth factor) expression levels correlate with the malignant transformation of the cervix [23]. Abnormal matrix metalloproteinases expression has also been suggested, with subsequent alteration of tissue architecture [24]. Last, as Woodby stated, “Cancers are not simply masses of proliferating cells. Rather, cancer acts like a dysregulated organ with a complex array of interactions between epithelial cells and fibroblasts, macrophages, endothelial cells and immune cells in the stromal microenvironment. The role of stromal cells and their products in cancer development is becoming more fully appreciated” [25].

Therefore, we estimate that our findings represent the expression of stromal changes associated with CIN, rather than of CIN itself. Our use of a plausibly more sensitive and less susceptible to variations technique might have led to this aspect being revealed.

Our results suggest that by using an ED as a reference material, transvaginal RTE is an excellent method for distinguishing between a normal cervix and CC, with a 100% sensitivity and a 94,9% specificity at a cut-off of 1.42. It should be highlighted that these results have been obtained after excluding the two cases of cancer complicated with hemorrhagic necrosis, in which aberrant strain results were noted. The technique also shows promising results in distinguishing between normal and CIN at a cut-off of 1.03, with a sensitivity and specificity of 75% and 74%, respectively. However, the above cut-offs only apply for the silicone type that we used as a reference material and for the specific machine in this study.

Certain limitations of this study need to be addressed. All imaging examinations were performed by a single observer; intra- and interobserver reproducibility studies have not been carried out. However, at least two studies reported good intra- and interobserver reproducibility of RTE of the cervix [11,26]. The small number of subjects, especially for the CC group, is another limitation. It is debatable if, by repeating the experiment on another equipment from another manufacturer, the cut-off values would remain the same. Another limitation of the method, irrespective of the ultrasound system used, represents those cases of CC complicated with hemorrhagic necrosis, in which the assessment of stiffness is compromised.

In **conclusion**, RTE SR of the uterine cervix, performed with a silicone ED as a reference material, seems a reliable method for distinguishing between a normal cervix and malignancy. The technique also shows promising results as a complementary investigation in diagnosing CIN. However, SR becomes inoperant in cases of cancer complicated with hemorrhagic necrosis. Larger studies are required to confirm the observations in this paper, as well as to evaluate CIN according to severity.

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Conflict of interest: none

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