SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

THE EFFECT OF GENITOURINARY SYNDROME OF MENOPAUSE ON THE VAGINA AND ITS TREATMENT POSSIBILITIES

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1. INTRODUCTION AND AIMS

As in developed countries, the aging process of the Hungarian population has been increasing rapidly. If we compare the population data published by the Hungarian Central Statistical Office in 2021 with the expected numbers in 2050, it is clear that the age pyramid shows the shape of a declining and aging society. As life expectancy has expanded, the need for a better quality of life has increased. This need must also be followed by medical care, as the treatment of diseases that have not received much attention so far is becoming essential. The prevalence of vulvovaginal atrophy during menopause, contemporarily called genitourinary syndrome of menopause (GSM), is increasing with age. The disease can also affect the urinary tract besides the lower genital tract, making its negative impact on quality of life even more pronounced. As a result, more and more women are asking for help. Because of the diversity of the disease, it is still difficult to establish a diagnosis, but many new methods and treatment options are available.

Our studies aimed to investigate the effect of GSM on the vaginal wall and its possible treatments. We aimed to evaluate the value of vaginal ultrasound elastography as a new diagnostic tool for GSM. According to our hypothesis, the Elastographic Index (EI) correlates with Meisels maturation index, as the gold standard, in the detection of GSM. Our further objective was to verify that the Elastographic Index (EI) is independent of menopausal status, and the number of previous vaginal births, and its value is lower in vaginal atrophy as measured by ultrasound elastography.

The treatment modalities of GSM/VVA are an intensively studied part of menopausal medicine, however many questions remain open in the field of both pharmacological and non-pharmacological treatment options. Although FDA-approved fractional CO_2 laser treatment is gaining popularity and there seems to be a consensus that it has a tissue remodeling effect, the

exact mechanism of action is unknown. It is a well-known fact that zinc is essential for vaginal tissue remodeling, hence we aimed to investigate the levels of zinc and copper in the cervicovaginal lavage after fractional CO_2 laser treatment.

2. MATERIALS AND METHODS

2.1. VAGINAL WALL ULTRASOUND ELASTOGRAPHY

2.1.1. STUDY DESIGN AND STUDY POPULATION

This prospective cohort study was carried out at the urogynecology outpatient clinic of the Department of Obstetrics and Gynecology, University of Debrecen, Hungary. Participants willing to contribute were included in the study either with or without the presence of vaginal dryness. Participants with any history of local or systemic hormone therapy within the past 6 months, vaginal infection at presentation, pregnancy, recent use of any over-the-counter vaginal product, cytological atypia, pelvic organ prolapse (POP) > stage 2 according to the POP quantification system (POP-Q) or severe urinary or fecal incontinence or any disease, which would interfere with the study protocol were excluded from the study. POP was assessed by a standardized POP-Q examination after the International Continence Society recommendations. To be considered in menopause individuals had to have at least 12 consecutive months of amenorrhea without any other reason, consistently elevated follicle-stimulating hormone (FSH) blood levels of 30 mIU/mL or higher or previous history of surgical removal of both ovaries. Women enrolled in the study underwent a detailed pelvic examination, vaginal cytology was obtained, and transvaginal elastography was performed. The clinical data gathered included components of the VHI score: elasticity, fluid secretion, pH, epithelial mucosa (integrity), and moisture components. Each component is scored on a scale of 1 (worst) to 5 (best). Lower scores indicate more severe atrophy. Demographic and pertinent clinical information was recorded prospectively and stored in a dedicated database. Our study was approved by the Hungarian National Institutional Review Medical Research Council (IRB Approval No 7239-3/2017/EUIG). All women signed written informed consent before participating in our research.

2.1.2. VAGINAL WALL ELASTOGRAPHY

All procedures were performed by a single physician (B.K.) with 10 years of experience in transvaginal ultrasonography (US) and two years of specialization in elastography. All participants have examined with a Voluson S10 (GE Healthcare, Zipf, Austria) transvaginal convex transducer in the dorsal lithotomy position. The transvaginal transducer was placed in the middle of vaginal length (calculated from total vaginal length measured during POP-Q examination), usually 4 to 5 cm cranial direction from the hymenal ring with light compression. Before elastography, conventional B-mode US was performed. Elastography measurements were carried out as described in previous studies.15-19 The system was switched to elastographic mode, and the elastogram map was placed over the entire thickness of both the anterior and posterior vaginal walls. After adequate frames of elastographic images were obtained, the strain image was displayed in a spectrum of colors from blue (hard) to red (soft) on the screen along with the 2-dimensional grey-scale US image. We generated the elastographic image after image stabilization when the pressure indicator turned green and the image was homogeneous and free of any artifacts. The image file obtained from the ultrasound machine was a JPEG file of 1136 x 852 pixels.

2.1.3. IMAGE ANALYSIS

All imaging analyses were performed prospectively by another physician (K.P.) who was blinded to the demographical and clinical characteristics of the participants. Considering the lack of universally accepted criteria for measuring vaginal elasticity with USE in the published literature, we set out a new methodology. Quantifiable elastographic parameters were measured with FemUhTool 1.0 (Fempharma LLC, Debrecen, Hungary), a semiautomatic image analyzer software, specially developed to analyze elastographic images. The software calculates the

Color Scale Index (CSI) within a designated Region of Interest (ROI). CSI was ranging from -255 to 255. If the analyzed ROI contains only blue pixels, the value of CSI is -255 and 255 if ROI contains only red pixels. 0 value represents the green color. The diameter of ROI was set to 21 pixels. Nine ROIs cover all the contacting surfaces of the anterior and posterior vaginal wall to the vaginal US transducer. The EI is the average of the nine ROI's CSI and represents the elasticity of the vaginal wall.

2.1.4. VAGINAL CYTOLOGY

Vaginal smear samples were collected in a single scraping of the middle third of the lateral vaginal wall with a spatula; independent board-certified cytopathologists, who were blinded to the clinical information, analyzed 200 cells per specimen. Parabasal cells (P), intermediate cells (I), and superficial cells (S) were counted. Superficial cells were assigned a point value of 1.0, intermediate cells were assigned a point value of 0.5, and parabasal cells were assigned a point value of 0. The number of cells in each category was multiplied by the point value, and the three results were added to arrive at VMV.8 A VMV value of 0 to 49 indicated a low estrogen effect, consistent with vaginal atrophy. Vaginal atrophy was defined as a VMV of less than fifty.

2.1.5. STATISTICAL ANALYSIS

The normality of continuous variables was examined using normal quantile plots. Two data sets (Atrophic group vs nonatrophic group) were compared with unpaired t-test (without or with Welch correction) or Mann-Whitney U test, in the case of normal distribution (with equal or unequal variances) or non-Gaussian distribution, respectively. For assessing the normality of

data D'Agostino-Pearson test was used. Descriptive statistics were reported as mean and standard deviation (SD) for continuous variables and frequencies and percentages for all categorical variables. To examine the bivariate associations between EI and age group, menopausal status, prior vaginal delivery, the number of vaginal deliveries, and vaginal atrophy (VMV < 50), a simple correlation analysis was used, and the Pearson Correlation Coefficient was calculated. Multiple linear regression was calculated to predict EI based on age, VMV, and the presence or absence of vaginal atrophy, in which the EI was the dependent variable, age, VMV, and the presence or absence of vaginal atrophy were independent variables. Unpaired Student t-tests and Pearson correlation analysis were performed using SAS Version 9.4 statistical software (SAS Institute Inc., Cary, NC), with the risk of Type I error set at a ¼ 0.05, D'Agostino

normality test and multiple linear regression model were carried out with IBM SPSS Statistics for Windows Version 25.0 statistical software (IBM Corp., Armonk, NY).

2.2. THE EFFECT OF CO_2 LASER TREATMENT ON VAGINAL ZINC AND COPPER LEVELS

2.2.1. STUDY DESIGN AND STUDY POPULATION

We enrolled women into our prospective cohort study at the outpatient urogynecology clinic of the Department of Obstetrics and Gynecology, University of Debrecen, Hungary, between 6/2017 and 6/2018. Postmenopausal women with the chief complaint of vaginal dryness were asked to participate in the study. We defined postmenopausal status if patients had at least 12 continuous months of amenorrhea without any other evident reason or permanently elevated follicle-stimulating hormone (FSH) blood levels (≥ 30 mIU/mL). Exclusion criteria were

pregnancy, hormone therapy (local or systemic) within the past six months, concurrent vaginal infection, cytological atypia, dysmenorrhea, POP > Stage 2, according to the pelvic organ prolapse quantification system [POP-Q], severe urinary or fecal incontinence (FI) or any disease which would influence the study protocol. Also, patients were asked to refrain from vaginal intercourse for three days before and two weeks after each treatment. At the first general gynecological visit, a medical history was taken (age, BMI, previous deliveries, menstruation cycle, the onset of menopause, hormonal therapy). The participants underwent 3 intravaginal microablative CO_2 laser therapy 4 weeks apart and were asked to mark the severity of their symptoms for vaginal dryness on a 0–10 Visual Analogue Scale (VAS) at the following timepoints: "baseline" before the first treatment; after the 1st treatment (right before the 2nd treatment); after the 2nd treatment (right before the 3rd treatment), and six weeks after the final, 3rd treatment of CO2-Laser system (SmartXide2V2LR, MonaLisa Touch®, DEKA, Florence, Italy). A score of 0 indicated the absence of a symptom and a score of 10 was the worst possible symptom. The clinical evaluation was performed by a board-certified obstetrician and gynecologist blinded to the specific study-related information. The clinical data gathered included components of the Vaginal Health Index Score (VHI): elasticity, fluid secretion, pH, and epithelial mucosa integrity and moisture components. Each component is scored on a scale of 1 (worst) to 5 (best). Lower scores indicate more severe atrophy. VHI was calculated at each time point, similarly to the VAS. Demographic and pertinent clinical information was recorded prospectively and stored in a dedicated database. Our study was approved by the Hungarian National Institutional Review Medical Research Council. All women signed written informed consent before participating in our research. There were no withdrawals or discontinuation of treatment due to adverse events.

2.2.2. LASER THERAPY

For laser treatment, a microablative, fractional CO_2 laser system (SmartXide2V2LR, Deka, Florence, Italy) was utilized, with a specific 360-degree probe designed for intravaginal procedures. Laser beams were fractionally emitted in small points (DOTs) around the vaginal mucosa during treatment. To achieve the required effect, the laser was used in D-Pulse mode. Depth was set, laser power, dwell time, and spacing were adjusted: Smart-Stak 1, 30 watts power, 1000 μs dwell time and 1000 μm spacing.

2.2.3. CERVICOVAGINAL LAVAGE

The cervicovaginal lavage fluid was collected during each visit, ensuring the absence of recent sexual activity or vaginal examination within three days. For sampling, the selected patients were in the lithotomy position, and a single-use plastic speculum was applied to open the vagina. After that, 10 ml of sterile 0.09% NaCl solution were injected into the vagina, trying to rinse as much mucosal surface as possible. The lavage fluid was introduced into the vagina with a plastic syringe for 60 s with three successive aspirations and emptied on the vaginal walls and cervix. The total amount of wash fluid is then recovered from the posterior fornix by syringe aspiration. CVL fluid was stored in plastic test tubes at − 80 °C until analysis.

2.2.4. SAMPLE PRE-TREATMENT

After volume measurement, the fluid samples of 5 ml were transferred without loss into 50 ml glass beakers by washing out the storage test tubes with 2 ml 65% (m/m) analytical pure nitric acid (Sigma-Aldrich, USA). These were then dried completely on an electric hot plate. An additional volume of 4 ml nitric acid was added to the samples with continued heating to

eliminate the organic matter until dryness. After cooling back to room temperature, an additional 1.00 ml of 30% (m/m) analytical pure hydrogen peroxide (Sigma-Aldrich, USA) and 1.00 ml of ultrapure water (MilliQ, Millipore System, Merck, Germany) was added to finalize the oxidation of remaining organic materials. The resulted dried samples first were diluted with 5 ml of ultrapure water (MilliQ, Millipore System, Merck, Germany) and then transferred into volume calibrated plastic test tubes with the help of an ultrasound bath and filled up to 10.00 ml with 0.1 M nitric acid and stored at 4 °C in a refrigerator until measurement. The purity of acids was verified by digesting blank samples containing only the chemicals but no samples. All samples were kept in polypropylene tubes at 4 °C until analysis.

2.2.5. ELEMENTAL ANALYSIS

The zinc and copper concentrations of the pre-treated fluid samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES 5100, Agilent Technologies, USA). The measurements were conducted in SVDV (Synchronous Vertical Dual View) mode, gaining intensity data from the axial and radial view, simultaneously. We applied an automatic sample introduction (SPS 4, Agilent Technologies, USA), and measured the samples in a randomized design. We performed measurements to generate a five-point calibration curve for the quantitative analysis of copper and zinc. Calibration solutions were diluted from a multielement standard of 1000 mg/L (ICP standard IV, Merck, Germany) with 0.1 M nitric acid in ultrapure water. We expressed the trace element concentration of vaginal fluid samples in mg/L.

2.2.6. STATISTICAL ANALYSIS

The statistical analysis was performed with SigmaStat/SPSS (SPSS Inc., Chicago, IL) software. To describe the clinical and demographic characteristics, means and standard deviations were used for continuous variables. Wilcoxon rank-sum test was used to compare the differences between the baseline scores and scores after subsequent treatments. Differences were considered significant when the P-value was less than 0.05. Data are presented as mean values (± standard deviation, SD) if not otherwise specified.

3. RESULTS

3.1. VAGINAL WALL ULTRASOUND ELASTOGRAPHY

Thirty women participated in our study. Based on the calculated VMV, participants were divided into two groups: (I) Atrophic group when the VMV was less than 50 and (II) Nonatrophic group when VMV was greater than or equal to 50. There were no significant differences in parity, the menopausal state of participants, and prior vaginal deliveries between the two groups. Women in the atrophic group were significantly older compared with nonatrophic women (mean ± SD, 66±8 vs 52±10 y, $P<0.01$). In the atrophic group, VMV and EI were significantly lower than in nonatrophic group (mean ± SD, 13±13 vs 66±14, $P<0.01$, and 20±21 vs 47±4, $P<0.01$). The VHI score was significantly lower in the atrophic group (mean ± SD, 9.4±2.011 vs. 16.6±4.22, $P<0.0001$). We found a moderate negative correlation between EI and age ($R= -0.495$, $P<0.01$; 95% CI: -0.7259 to -0.1642), a moderate negative correlation between EI and VMV ($R= 0.574$; $P= 0.0009$; 95% CI: 0.2707 to 0.7747), and a strong negative correlation between EI and vaginal atrophy ($r= -0.706$, $P<0.0001$; 95% CI: 0.8501 to -0.4639) in the correlation analysis. Multiple linear regression was calculated to predict EI based on age, VMV, and the presence or absence of vaginal atrophy. A significant regression equation was found $F(3,26)=9.816$, $P<0.0001$, with an $R2$ of 0.531. The multiple linear regression model showed that only the presence or absence of vaginal atrophy is a significant predictor of EI ($P=0.004$, 95% CI: -55.215 to -11.602).

3.2. THE EFFECT OF CO_2 LASER TREATMENT ON VAGINAL ZINC AND COPPER LEVELS

Twenty-nine postmenopausal women with the chief complaint of vaginal dryness were enrolled in our study. The average age was 58.24 ± 8.60 years, and on average 11 ± 8 years had passed

since their last menstrual period. Clinical evaluation revealed no other apparent reason for the vaginal dryness other than menopausal vaginal atrophy. We have found a strong negative correlation between VAS and VHI ($r = -0.681$, $P < 0.01$). The VHI improved significantly after each treatment compared to the baseline (mean ± SD VHI score, 13.03 ± 4.49 before vs. 15.55 ± 4.35 after the 1st, 17.79 ± 4.57 after the 2nd, and 19.38 ± 4.39 after the 3rd treatment, $P < 0.01$). The patient-reported VAS vaginal dryness score was significantly lower after each laser treatment (mean ± SD VAS score, 6.59 ± 2.86 before vs. 4.17 ± 2.86 after the 1st, 2.45 ± 2.43 after the 2nd, and 1.41 ± 1.94 after the 3rd treatment, $P < 0.01$). CVL zinc levels were significantly higher compared to copper levels at baseline (mean ± SD, mg/L, 0.06 ± 0.04 vs. 0.006 ± 0.006, $P < 0.01$). The first laser treatment had no significant effect on the CVL zinc levels. After the second laser treatment, CVL zinc levels were significantly higher, but after the 3rd treatment, CVL zinc levels returned to the baseline values. Contrary to zinc levels, copper levels in the CVL of women undergoing vaginal CO_2 laser remained similar after three vaginal laser treatments.

4. DISCUSSION

The aging of the population has been increasing rapidly worldwide in well-developed countries, also as Hungary. According to the data from the Hungarian Central Statistical Office in 1990 the mean age of the woman was 39 years, which is increased to 45 in 2021. Today, 20 percent of the population is above 65 years in Hungary. The prevalence of menopause-linked issues and also pelvic floor diseases is increasing with the aging of the population. Patients' need for a high quality of life and the severe negative effect of various menopausal problems on quality of life results in an increasing number of visits to urogynecology care units.

The genitourinary syndrome of menopause (GSM), or previously vulvovaginal atrophy (VVA), includes all the abnormalities of the vulva, vagina, and urethra and consequent symptoms that appear as the result of decreased levels of circulating estrogens and other sex steroid hormone levels. GSM/VVA has a high impact on a woman’s quality of life and sexual function, with a prevalence of 40% to 57% after menopause. This term describes various menopausal symptoms and signs such as pruritus vulvae, dryness, and burning, vaginal pain, stenosis of the vagina and introitus, decreased elasticity, sexual dysfunction, and urological symptoms, for example, incontinence, nocturia, dysuria.

In menopause diminished estrogen level leads to morphological and secretory changes in the vulva and vagina. Also, due to decreasing blood supply lubrication and elasticity deteriorate. Natural aging causes a reduction in glycogen production that has a continuous negative effect on the vaginal epithelium facilitating the development of GSM.

GSM is a clinical diagnosis based on medical history and pelvic examination. More specific methods are also available to support the diagnosis, assess the efficacy of the treatments, and for research purposes. In research studies, a common method for diagnosing vaginal atrophy is to calculate the vaginal maturation value (VMV) of Meisels obtained from vaginal cytology.

Cytology is a relatively cheap, non-invasive method but requires a cytology laboratory. Vaginal atrophy is defined if the VMV is less than 50. Vaginal Health Index (VHI) as an objective measurement could also aid in the diagnosis of GSM/VVA. In addition, a recent study revealed that the amount of zinc in cervicovaginal lavage could be used as a marker for vaginal atrophy. Explicit, yet invasive, methods to investigate the changes in vaginal wall composition are full-thickness samples or biopsy. To date, no sonographic technique has been developed to diagnose vaginal atrophy.

Ultrasound elastography (USE) is a parametric imaging technique that allows quantification of the elasticity of tissue. Currently, available USE techniques can be classified by the measured physical quantity into strain and shear wave imaging. USE has promising results for assessing liver fibrosis, breast lesions, thyroid nodules, renal fibrosis, or prostate masses. The importance of this new method expands rapidly in the field of obstetrics and gynecology also. Ultrasound elastography has a promising role in the diagnosis of endometriosis and adenomyosis and helps to differentiate benign and malignant cervical and ovarian lesions. The use in the prediction of the outcome of labor induction and preterm birth, and in the evaluation of preeclampsia are appearing. A new application for the assessment of pelvic floor biomechanics is emerging. Many studies suggest that USE can be useful for quantifying vaginal wall elasticity, but its role in the diagnosis of GSM/VVA is unknown.

To our knowledge, this study is the first study to describe a correlation between the elasticity of the vaginal wall measured by USE and vaginal atrophy. In our prospective cohort study, we found a significant association between EI, representing the elasticity of the anterior and posterior vaginal wall, and participants' age, VMV, and the presence or absence of vaginal atrophy. However, multiple linear regression models showed that among the mentioned significant factors only vaginal atrophy (VMV<50) is a significant predictor of EI.

Our study demonstrates that it is feasible to apply vaginal wall elastography in clinical practice, and because modern ultrasound systems are already equipped with elastography, this method will be commonly available. It was clear that the elasticity of the vaginal wall was age-related; nevertheless, after fitting the multiple regression model, age was not a significant predictor of EI. One of our study's strengths was that we are the first to investigate the association between strain elastography measurements and vaginal atrophy. We used a non-invasive, easy to perform method to establish the diagnosis of VVA, a main component of GSM. Our study has several weaknesses, which need to be offset and improved in a further study. First, the number of participants was low, and the results need additional testing on a larger sample. Second, it was a single-center study, and to confirm our results a multicenter, large-scale study would be required. Third, a potential limitation of this study is the lack of standardized examination settings, which is also required to test the clinical usefulness of this new diagnostic tool.

In conclusion, our trial has shown that strain USE has a potential clinical value in diagnosing vaginal atrophy. Taking into consideration the relatively small sample size as a limitation of this study, our preliminary data need to be confirmed by larger clinical trials. We believe that vaginal strain elastography could become a new sonographic method aiding in the diagnosis of vaginal atrophy.

Besides developing a new diagnostic tool, our research group aims to understand the underlying mechanism of the available treatment modalities for GSM/VVA. Estrogen is the only Food and Drug Administration (FDA) approved treatment of vulvovaginal atrophy. For women who have an aversion to hormonal therapy over-the-counter (OTC) products and lubricants can be offered to relieve their symptoms. Recently several publications reported on the beneficial effect of vaginal laser therapy on vaginal mucosa in VVA, vaginal health and flora, sexual function and

dyspareunia, and urinary incontinence, although the FDA has not approved vaginal laser treatment for this indication.

The management varies according to symptom severity. First-line therapy contains local non-hormonal (lubricants, moisturizers) and hormonal products, in severe cases, systemic hormone replacement therapy (HRT), or selective estrogen receptor modulators (SERMs) can be offered. Fractional CO2 laser is widely used in surgery and dermatology. This form of therapy proved to be safe and efficient by inducing tissue remodeling resulting in the rejuvenation of the skin. Similar changes are detectable in vaginal tissue after CO2 laser treatment. As a result of the thermal effect of the laser beam first collagen denaturation and tissue shrinking occurs, then rapid regeneration begins with the synthesis of new extracellular matrix (ECM) components and epithelial proliferation. Fractional CO2 laser rejuvenation can be delivered with a variety of microbeam sizes and densities to achieve the required effect without the thermal damage to the surrounding tissue.

Zerbinati et al. demonstrated an increased number of active fibroblasts, glycogen-rich cells, and augmented content of extracellular matrix (ECM) elements such as elastin and collagen in vaginal mucosa after laser treatment. Salvatore et al. described similar changes in the vaginal tissues in response to treatment. Other authors investigating postmenopausal vaginal cytology after laser treatment found significant improvement in vaginal maturation values (VMV) and/or vaginal symptoms, which inversely related to atrophy. Athanasiou's study revealed that this form of therapy (laser) helps repopulate existing bacteria and restore normal premenopausal flora in the vagina. Besides, publications are reporting the beneficial effect of vaginal CO2 laser on vulvodynia and lichen sclerosis as well. The basic principle of its remodeling effect is that the laser therapy energy is absorbed by water in the treated tissue causing a cascade of events to happen. The CO2 laser beams are delivered fractionally, causing ablative micro-millimeter thermal damage. As a result, a rapid epithelial repair mechanism begins. In the short term, the

collagen fibers became thicker and shorter. After a while, neovascularization increased fibroblast activity, and newly formed collagen fibers are detectable in the epithelium. Previous studies illustrated the critical role of Zn supply in connective tissue formation. Based on these findings, we could presume that the collagen genesis and vaginal ECM remodeling induced by the thermal effect of CO2 laser occur more effectively in a zinc-rich environment.

Copper also plays an important role in connective tissue biosynthesis and physiology. Animal experiments on copper-deficient chicks and swines revealed histological evidence of abnormal elastic tissue in the aorta resulting in major vessel rupture. Rucker et al. demonstrated that copper deficiency resulted in decreased mechanical strength in tissues high in elastin and collagen (blood vessels, tendons, and bone) due to insufficient collagen and elastin cross-linking.

Although vaginal wall biopsies would be a more direct way to gather more information about the mechanisms maintaining the vaginal zinc and copper balance, the in vivo application of this invasive method raises ethical questions and limits specimen collection. Previous studies revealed that the cervicovaginal lavage (CVL) is a useful way to collect a sample from the female lower genitourinary tract. The content of the cervicovaginal lavage fluid (CVL) accurately reflects the physiological changes of the vagina and cervix during pregnancy or menopause and can detect pathogens, cervicopathological changes, and the presence or absence of different proteins and minerals during several genital diseases. This suggests it could be a useful surrogate for vaginal biopsy. Though the exact pathways and mechanisms behind the zinc and copper transport from the vaginal epithelial tissue to the cervicovaginal fluid (CVF) are still under investigation, there is likely a correlation between the level of these elements in vaginal tissue and cervicovaginal lavage (CVL).

To our knowledge, we are the first to investigate zinc and copper levels in the cervicovaginal lavage (CVL) after CO2 laser treatment. Fractional CO2 laser treatment of the vagina affected

CVL zinc and copper levels differently. While CVL copper levels were not different after each laser treatment, zinc levels were significantly higher after the second treatment before returning to baseline values after the third laser treatment. Based on our findings, we conclude that the extracellular matrix (ECM) regeneration and repair mechanisms in the vaginal epithelium induced by the CO2 laser treatment require increased tissue zinc concentration, and the elevated CVL zinc concentration detectable after CO2 laser therapy reflects this increased need for zinc.

We believe that the strength of our study is that this is a new perspective, bringing novel information to the existing literature. The primary weaknesses of our study are the relatively small sample size and lack of a control group. A future trial designed with a laser treatment arm and a sham treatment arm would provide more critical data. Besides, full-thickness vaginal wall biopsies rather than CVL as a surrogate marker would give conclusive results on vagina tissue levels of zinc and copper in response to treatment

In conclusion, fractional CO2 laser treatment of the vagina significantly improved the symptoms of vaginal dryness in postmenopausal women. In addition to subjective improvement, the VHI has improved significantly as well. Laser treatment affected the levels of zinc and copper in CVL differently. While CVL copper levels were not different after each laser treatment, zinc levels were significantly higher after the second treatment before returning to baseline values. The fact that zinc and not copper levels changed in the CVL, suggests that zinc may play a stronger role in the remodeling process observed with laser treatment. Further studies are required to explore zinc's role in the CVL.

GSM/VVA causes a high burden on patient care physicians in the aging societies of well-developed countries. Keen interest in good quality of life by patients puts a challenge on researchers to develop new diagnostic and treatment approaches. This thesis aims to add new findings to the field of urogynecology in the presented topics.

5. SUMMARY OF MAJOR RESULTS AND SCIENTIFIC NOVELTIES

1. To our knowledge, we conducted the first study to describe a correlation between the elasticity of vaginal wall measured by Ultrasound Elastography and vaginal atrophy.
2. We invented the Elastography Index (EI), representing the elasticity of the anterior and posterior vaginal wall.
3. We found a significant association between EI, and participants' age, VMV, and the presence or absence of vaginal atrophy.
4. In our multiple linear regression model showed that among the mentioned significant factors only vaginal atrophy (VMV<50) is a significant predictor of EI.
5. Our study has shown that vaginal strain elastography could become a new sonographic method aiding in the diagnosis of vaginal atrophy.

6. To our knowledge, we are the first to investigate zinc and copper levels in the cervicovaginal lavage (CVL) after CO_2 laser treatment.
7. Fractional CO_2 laser treatment of the vagina affected significantly the CVL zinc level, but not the cooper level.
8. Our study provided novel information about the zinc and cooper levels after multiple vaginal laser treatment: zinc levels were significantly higher after the second treatment before returning to baseline values after the third laser treatment.
9. This is the first study investigating in one cohort the Vaginal Health Index Score, as an objective scale, the Visual Analogue Scale, as a relatively subjective scle with the changing of vaginal zinc and cooper levels after vaginal CO_2 laser treatment.
10. Our result suggests that zinc may play a stronger role in the remodeling process observed with laser treatment.

6. PUBLICATIONS

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Registry number: DEENK/102/2022.PL
Subject: PhD Publication List

Candidate: Krisztina Pákozdy
Doctoral School: Doctoral School of Nutrition and Food Sciences

List of publications related to the dissertation

1. Sipos, A. G., **Pákozdy, K.**, Jäger, S., Larson, K., Takács, P., Kozma, B.: Fractional CO2 laser treatment effect on cervicovaginal lavage zinc and copper levels: a prospective cohort study. *BMC Womens Health. 21* (1), 235, 2021.
   DOI: http://dx.doi.org/10.1186/s12905-021-01379-1
   IF: 2.809 (2020)
2. **Pákozdy, K.**, Sipos, A. G., Bombicz, M., Lampé, R., Póka, R., Takács, P., Kozma, B.: Vaginal elasticity is significantly decreased in vaginal atrophy. *Menopause. 27* (12), 1420-1424, 2020.
   DOI: http://dx.doi.org/10.1097/GME.0000000000001673
   IF: 2.953

List of other publications

3. Kozma, B., **Pákozdy, K.**, Lampé, R., Berényi, E., Takács, P.: Ultrahang-elasztográfia alkalmazásának lehetőségei a szülészet-nőgyógyászatban. *Orv. hetil. 162* (18), 690-695, 2021.
   DOI: http://dx.doi.org/10.1556/650.2021.32094
   IF: 0.54 (2020)

DEBRECENI EGYETEM Egyetemi és Nemzeti Könyvtár

Total IF of journals (all publications): 6,302
Total IF of journals (publications related to the dissertation): 5,762

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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