

School of Doctoral Studies

-summary of PhD thesis-

Title: Role of microRNA and elastography in the diagnosis of adenomyosis

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Introduction:

Benign uterine diseases are a range of gynaecological pathologies affecting women of all ages, especially those during the reproductive period. The most common benign uterine diseases include adenomyosis, endometrial polyps and leiomyomatosis or uterine fibromatosis. All these conditions heavily impact the quality of life of affected patients and often cause dysfunctional symptoms with impaired fertility. Dysfunctional symptoms include intermenstrual pelvic pain, dysmenorrhoea and abnormal uterine bleeding. For patients suffering from these diseases, infertility is both a consequence of mechanical complications and architectural and molecular changes produced by these disorders. Non-invasive diagnosis of these pathologies, especially of adenomyosis, is challenging and treatment of these conditions depends on the age and desire of the patient to preserve fertility. Thus, there is a need to increase the non-invasive diagnostic accuracy of adenomyosis and leiomyomatosis, in order to provide optimal early solutions for patients affected by these abnormalities. Therapeutic outcomes are closely related to early diagnosis of these conditions.

Objectives:

In the first retrospective study of 110 patients, the aim was to identify specific features of ultrasound semiology for the non-invasive diagnosis of primary and recurrent ovarian endometriomas.

The second, prospective study aimed to evaluate the value of transvaginal ultrasound combined with qualitative elastography in the diagnosis of adenomyosis. A secondary objective of the study was to assess the ability of the method to differentiate adenomyosis from leiomyomatosis using lesional stiffness, in those situations where sonographic semiology is not specific.

In the third, prospective study, we assessed the ability of transvaginal ultrasound combined with elastography to differentiate adenomatous tissue from leiomyomatosis in the cases of co-existence of these pathologies within the same uterine specimen. All data obtained from the study group were compared with a control group of patients with apparently healthy uterine tissue.

The fourth study, conducted prospectively, aimed to correlate gene expression between circulating microRNA and tissue microRNA, identified from specific lesions within

the surgical specimen. Secondly, this study aimed to identify whether the 2 studied microRNAs, miR-135a and let-7b, have the ability to distinguish adenomatous lesions from leiomyomatosis, based on different gene expression.

General methodology:

Out of the four studies included in this paper, one was retrospectively, while three were prospectively conducted. The retrospective study enrolled patients who were histopathologically diagnosed with ovarian endometriomas and undergoing surgical treatment from January 2007 to August 2019. Prospective studies included patients of reproductive ages (23-52 years) admitted in the Obstetrics-Gynecology clinic of Mureş Clinical County Hospital, enrolled in the study between May 2019 and November 2021. The patients included in the prospective studies experienced meno-metrorrhagia, chronic pelvic pain due to compression, dysmenorrhea and were suspected of benign uterine pathology. All patients required surgical treatment. Preoperatively, a transvaginal ultrasound combined with qualitative elastography was performed to determine the presence of benign tumour pathology and two 5 ml blood samples were collected to quantify serum expression of miR-135a and let-7b. Postoperatively, 3 tissue fragments were collected from suspicious lesional tissues for quantification of miR-135a and let-7b expression.

Results:

The first study showed that ultrasonographically suspected and histologically confirmed primary ovarian endometriomas were larger than ultrasonographically suspected and histologically unconfirmed primary ovarian endometriomas ($p=0.0098$). Also, histologically confirmed primary ovarian endometriomas had larger sizes than histologically confirmed recurrent ovarian endometriomas ($p<0.0001$), and lack of papillary projections was associated with a positive diagnosis of ovarian endometriomas ($p=0.0279$).

The second study showed that the mean and maximum strain ratio (SR) values obtained from qualitative elastography were significantly higher for adenomyosis than leiomyomatosis ($p<0.001$), and that transvaginal ultrasound in combination with qualitative elastography provided an increased sensitivity and specificity in the diagnosis of adenomyosis and leiomyomatosis.

In study three it was found that mean and maximum SR values obtained from qualitative elastography were significantly higher in adenomyosis compared to leiomyomatosis, and in both cases compared to normal tissue ($p<0.001$). Thus, qualitative elastography combined with transvaginal ultrasound represents a valuable diagnostic tool, with high sensitivity and specificity in the diagnosis of coexisting pathology.

The fourth study identified differences in serum and tissue let-7b-5p and miR-135a-5p expression between adenomyosis and leiomyomatosis, but with no statistically significant association. Tissue gene expression did not correlate with serum gene expression for the two microRNAs studied, with significant differences between the values quantified within tissue and blood.

General conclusions:

Ultrasonography has a high sensitivity and specificity in the diagnosis of primary and recurrent ovarian endometriomas. Qualitative elastography combined with transvaginal ultrasonography have proven as valuable tools in the diagnosis of adenomyosis using the lesional stiffness, which has the highest stiffness among the two studied benign uterine conditions, and with high diagnostic sensitivity and specificity. At the same time, qualitative elastography combined with transvaginal ultrasonography has also proved utility in the differential diagnosis of coexisting benign uterine disorders. Using lesional stiffness it was possible to identify adenomyosis and fibromatosis in the same uterine specimen, with high sensitivity and specificity. The studied microRNAs represented by let-7b-5p and miR-135a-5p cannot be used as biomarkers in the differential diagnosis of uterine adenomyosis and fibromatosis. Serum expression of let-7b and miR-135a did not correlate with tissue expression in neither of the 2 study groups with benign uterine pathology mentioned above.

Originality of the thesis:

This thesis contributed significantly to the literature by addressing a trending subject, its originality being related to the role of elastography and genetic markers in benign gynecological uterine pathology, in light of the increasing frequency of adenomyosis. Therefore, study 2 brings elements of innovativeness by demonstrating the ability to differentiate uterine fibromatosis from adenomyosis using qualitative elastography combined with transvaginal ultrasound. In study 3 we demonstrated the value of qualitative elastography and transvaginal ultrasound in diagnosing the coexistence of adenomyosis and leiomyomatosis within the same uterine specimen. Study 3 is the first study in the literature to consider this approach, which is another innovative and original element of the research. Study 4 examined whether certain microRNA molecules with a proven role in modulating cell proliferation, differentiation and apoptosis can be used as potential non-invasive diagnostic biomarkers of adenomyosis. Thus, we sought to analyse whether these biomarkers are differentially expressed between adenomatous and fibromatous tissue in both serum and tissue levels. The use of these markers in the differential diagnosis of these pathologies represents the third element of innovation and originality of this thesis.