Original Research

OBSTETRICS AND GYNECOLOGY

Retrospective study of the factors involved in the development of adenomyosis and the *in vitro* link between adenomyosis and breast cancer

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Abstract

Background and aims. Adenomyosis is a heterogeneous disease, which differs from patient to patient. The objective of our study was to evaluate the risk factors that influence the occurrence of adenomyosis, more precisely to highlight aspects that may be used in practice. In addition, the *in vitro* impact of levonorgestrel (a possible predisposing factor in the occurrence of adenomyosis) on MDA-MB-231 cells was evaluated, trying to obtain a link between adenomyosis and mammary cancer.

Methods. Clinical and demographic data of patients diagnosed with adenomyosis hospitalized between January and September 2023 in the Obstetrics-Gynecology Clinic were analyzed. For the *in vitro* assays, the MTT and LDH method was used to investigate the effect on cell viability and the potential cytotoxic effect of LG on MDA-MB-23 cells.

Results. Out of a total of 99 hysterectomies performed, the diagnosis of adenomyosis was confirmed by ultrasound in 28 cases. Among our patients, we could observe that most of cases of adenomyosis developed in women between 40 and 45 years old. Multiple pregnancies can influence the development of this uterine pathology, along with a history of uterine surgery and abortions. It was also found that treatment with sex hormones can increase the risk of adenomyosis. Our *in vitro* study has showed that LG stimulates the proliferation of MDA-MB-231 cells depending on the dose and time.

Conclusions. Personal history along with progestin treatment may influence myometrial lesions, leading to diffuse or focal adenomyosis. Moreover, *in vitro*, LG has been shown to stimulate the proliferation of breast cancer cells.

Keywords: adenomyosis, risk factors, levonorgestrel, MDA-MB-231 cells

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Introduction

Adenomyosis is a frequent benign uterine pathology in adult women, which is characterized by the presence of endometrial tissue (glands and stroma) in the myometrium. Women suffering from this condition may experience dysmenorrhea, abnormal uterine bleeding, pelvic pain or infertility, however, one third of cases are asymptomatic [1]. Until not long ago, the diagnosis of this disease could only be made retrospectively through the histological examination of the uterus, following hysterectomy. This pathology has become increasingly common in young women, and the new imaging techniques such as magnetic resonance imaging (MRI) and transvaginal pelvic ultrasound (TVUS) are taking steps towards diagnosing the disease, which is still little known [2].

The first information about the disorder appeared in 1860 when the German doctor Carl von Rokitansky noticed the presence of endometrial glands

in the myometrium [3,4]. Later, in 1925, Frankl used the term "adenomyosis", following that in 1972 the definition was reproduced by Bird et al. as "benign endometrial invasion into myometrium, diffusely enlarged uterus, ectopic, neoplastic endometrial glands, and stroma surrounded by hypertrophic and hyperplastic myometrium" [5].

The pathogenic mechanisms of adenomyosis are not fully elucidated, but in recent years it has been observed that inflammatory molecules, extracellular matrix enzymes, sex hormone receptors, as well as neuroangiogenic factors and growth factors play an important role [6]. Adenomyosis is considered to originate from the basal invagination of the endometrium into the myometrium, an invagination that occurs along the intramyometrial lymphatic system [7]. Another theory is that the condition may arise de novo from a metaplastic process starting from intramyometrial endometrial tissue [8].

Adenomyosis is a pathology characterized by hypertrophic or hyperplastic myometrium surrounding the stroma and glands, causing either diffuse or focal expansion of the uterus. Diffuse adenomyosis was defined as a widespread invasion of the basal glands in the myometrium, while focal adenomyosis was characterized by lesions in the inner, middle or outer myometrium, involving the posterior or anterior wall of the uterus [9]. The most diagnosed form of adenomyosis is the diffuse one, while the cystic variant, focal adenomyosis, is much rarer, found especially in women under 30 years of age [10].

In recent years, adenomyosis has transformed from a histopathological entity into a clinical condition, diagnosed by imaging techniques [11]. Transvaginal ultrasound has become the main analysis in the diagnosis of adenomyosis. Because adenomyosis is a gynecological condition that affects women of reproductive age, causing high rates of ovum implantation failure, pregnancy loss or premature birth [12], it has received special attention among medical specialists.

Adenomyosis-related cancer is extremely rare; it occurs only in 1% of cases, especially in elderly people, but it can also be due to this disease [13,14]. Adenomyosis and cancers at this level present common manifestations, but the incriminating pathogenic mechanism of malignant changes remains unknown. A knowledge of the process by which the disease develops helps diagnose and institute the correct therapy [15].

Sex hormone imbalance and inflammation are the most known factors that promote the invasion of the myometrium with endometrial cells or the modification of Müllerian remnants in adenomyosis [16]. These factors are also involved in benign and malignant breast pathologies [17]. Breast cancer is one of the most common malignant pathologies worldwide [18]. Breast cancer is a heterogeneous pathology, with different factors that play a role in its development, such as age, personal history, and exposure to sex hormones [19]. The implication of sex hormones in the development of breast cancer lies in their ability to stimulate cell division [20]. Moreover, a study showed that women who underwent progestin treatment had a higher risk of developing breast cancer compared to women who administered estrogen-only medication [21].

Currently, the causes of the development of adenomyosis are not fully known, and this research aims to collect evidence in this regard. Our study focuses on the evaluation of the symptoms and more precisely the risk factors that influence the development of adenomyosis; information that will strengthen knowledge about this increasingly common disease. Further, following the data obtained in the retrospective study, we aimed to evaluate *in vitro* the relationship between levonorgestrel, which can influence the occurrence of adenomyosis, and breast cancer.

Methods

Clinical data

Our clinical study was carried out over a period of 9 months (January-September 2023) in the Obstetrics-Gynecology Clinic of the "Pius Brinzeu" County Emergency Clinical Hospital in collaboration with the "Victor Babes" University of Medicine and Pharmacy. The protocol was approved by the Scientific Research Ethics Committee of the "Victor Babes" University of Medicine and Pharmacy.

The study method consisted in analyzing the following parameters: age, demographic data, symptoms, obstetric and gynecological antecedents - childbirths/abortions, associated pathologies and treatment with progestogens. The analyzed group included patients hospitalized between January and September 2023 in which the diagnosis of adenomyosis was confirmed in all cases by ultrasound. We therefore retrospectively analyzed all cases in which a hysterectomy was performed and adenomyosis was confirmed by ultrasound examination.

Patients included in the study were selected based on strict inclusion and exclusion criteria.

1. Inclusion criteria:

- patients admitted to the Obstetrics-Gynecology Clinic of the "Pius Brinzeu" County Emergency Clinical Hospital
- patients ≥ 18 years old
- confirmed diagnosis of adenomyosis

2. Exclusion criteria:

- patients with incomplete available data

In vitro data

Specific reagents

Levonorgestrel, trypsin-EDTA solution, and dimethyl sulfoxide (DMSO) were acquired from Sigma Aldrich, Merck KgaA (Darmstadt, Germany). The trypsin-EDTA solution, fetal bovine serum (FBS), penicillin/streptomycin combination, and the specific cell medium, Dulbecco's Modified Eagle's medium (DMEM) were procured from Pan-Biotech GmbH (Aidenbach,

Germany). The MTT (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide) kit was purchased from Roche Holding AG (Basel, Switzerland), and the lactate dehydrogenase (LDH) kit was bought from ThermoFisher Scientific (Waltham, MA, USA). All reagents utilized were of high analytical standard purity. The MDA-MB-231 (ATCC HTB-26™) breast cancer line was purchased from ATCC (American Type Cell Collection, Lomianki, Poland).

Cell culture

The *in vitro* analyses were carried out on the MDA-MB-231 breast adenocarcinoma cell line grown in Dulbecco's Modified Eagle's medium supplemented with 10% FBS, and 1% penicillin/streptomycin (100 U/mL/100 µg/mL). The cells were cultured and kept under standard conditions (5% CO_2 and 37 °C) in a humidified atmosphere.

Viability assay

Cell viability was analyzed by the MTT assay. MDA-MB-231 cancer cells were seeded in 96-well plates (1×10^4 cells/100 µL/well) and were stimulated with two concentrations of levonorgestrel (LG) solution (0.5 µM and 15 µM) in DMSO for 24 and 48 h. After the stimulation period, 100 µL of fresh media and 10 µL of tetrazolium salt solution (10 µL) were added to each well and incubated for 3 h. Lastly, 100 µL of solubilization buffer was added for 30 min, at room temperature, without light. The absorbance values for reduced MTT were read at 570 nm using Cytation 5 from BioTek Instruments Inc. (Winooski, VT, USA).

Cytotoxicity test

To complement the results of the MTT test, a possible cytotoxic effect of LG on MDA-MB-231 cells was investigated using the LDH method. Therefore, the cells were grown under the same conditions as for the MTT method (1×10^4 cells/100 µL/well) and then the cells were stimulated with the two concentration of LG solution for 24 h and 48 h, respectively. After the stimulation period, a volume of 50 µl from each well was dispensed into a new plate (96 wells), on top of which 50 µL of the reaction mixture was placed for 30 min at room temperature. Finally, 50 µL of stop/well solution was added. Absorbances were measured at 490 and 680 nm, respectively, using Cytation 5 from BioTek Instruments Inc.

Statistical analysis

In vitro results are expressed as means ± standard deviation (SD). The software GraphPad Prism version 9.4.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for data interpretation. One-way ANOVA test and Dunett's multiple comparisons post-test were conducted to compare the results. The statistically significant differences between results were marked with * (** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$).

Results

Clinical data

To begin with, our study aimed to identify as much objective data as possible, to understand the predisposing factors in the development of adenomyosis, which can be helpful in future cases. It should be remembered that the treatment in the case of adenomyosis is surgical, and the dominant symptoms of this condition are often common to other pathologies that are easier to diagnose and which, in most cases, have other therapeutic methods.

The group of patients analyzed, during the 9 months of the study, includes 99 patients who underwent hysterectomy interventions. Out of a total of 99 hysterectomies performed during this period, the diagnosis of adenomyosis was confirmed by ultrasound in 28.28% of cases (Table I). The other hysterectomies were performed for different pathologies, mainly for uterine fibroids, uterine prolapse and atypical endometrial hyperplasia.

Figure 1 presents the analysis of the incidence of hysterectomies during the study period and highlights the prevalence of the diagnosis of adenomyosis from these interventions. Following the graphic representation, we can observe a Gaussian distribution of the diagnosis of adenomyosis, with most cases being observed in April.

The clinical observations, the enlarged uterus with dysmenorrhoea and menorrhagia, suggest adenomyosis, but a clear diagnosis is needed, and an initial step is done through the ultrasound examination. Ultrasound is the most used technique in the diagnosis of gynecological pathologies because it is easily available in the outpatient clinic, it is relatively less expensive than other imaging techniques.

In our study, we evidence the changes observed in a 40-year-old patient diagnosed with diffuse adenomyosis. More precisely, there were differences in the thickness of the anterior wall compared to the posterior one, a mixed sandy appearance, and possibly also cystic adenomyosis in the thickness of the anterior wall, as can be seen in figure 2. In addition, the patient had severe menstrual pain and prolonged menstruation.

Table I. Diagnosis on admission.

	Admission diagnosis				
	Adenomyosis	*Malignant tumor*	*Uterine fibroid*	*Polyfibromatous uterus*	*Other diagnosis**
Patients (n)	28	21	19	16	15
Patients (% of total)	28.28	21.21	19.19	16.16	15.15

*Table note: *Other diagnostics include: cervical dysplasia (n=5), metrorrhagia (n=4), urinary infection (n=3), iron deficiency anemia (n=3)*

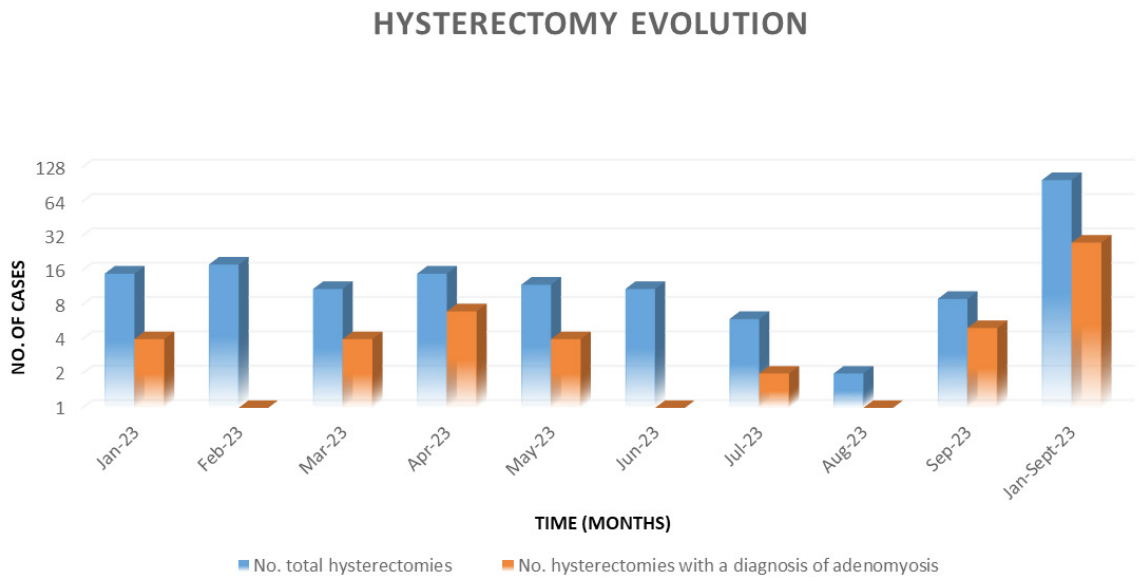

Figure 1. The evolution of hysterectomy cases.

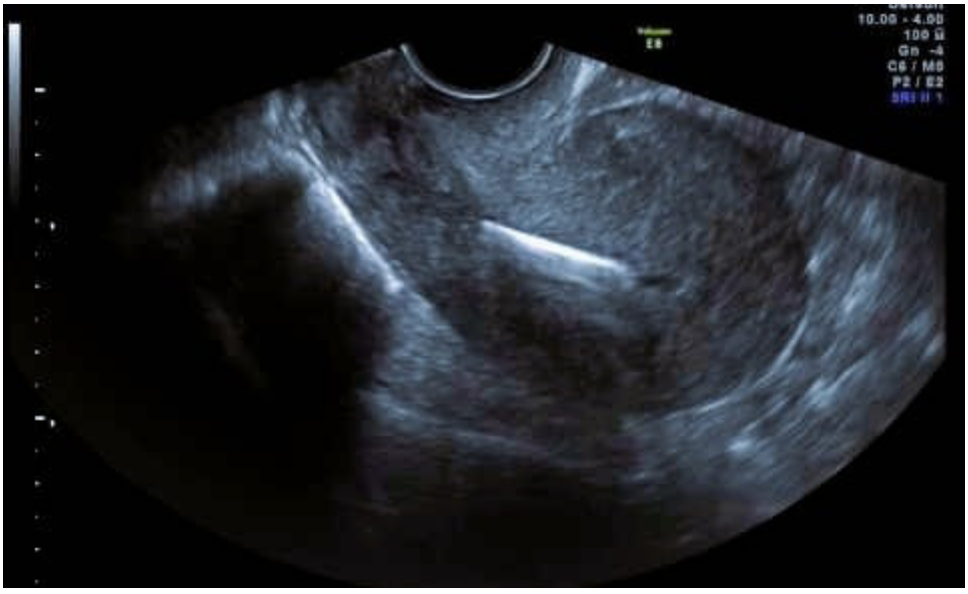

Figure 2. Changes in diffuse adenomyosis.

The next two images (Figure 3a. and Figure 3b.) are from another patient, diagnosed with focal adenomyosis, in which the difference in thickness between the anterior wall and the posterior uterine wall, the hypervascularization of the uterine walls, the mixed sandy ultrasound appearance and the change in the trajectory of the endometrium can again be observed. The linear appearance of the uterine cavity changes into an S-shaped appearance due to the infiltration of the endometrial glands at the level of the myometrium. In addition to the clear appearance of adenomyosis, the presence of two intramural fibromatous nodules can be noted, which may have a component of cystic adenomyosis.

In image 3a, the difference in thickness between the anterior and posterior uterine wall can be seen, and this is also confirmed in image 3b with vascular Doppler. Furthermore, the ovaries cannot be visualized, a proof that the pathology has not extended to the ovaries.

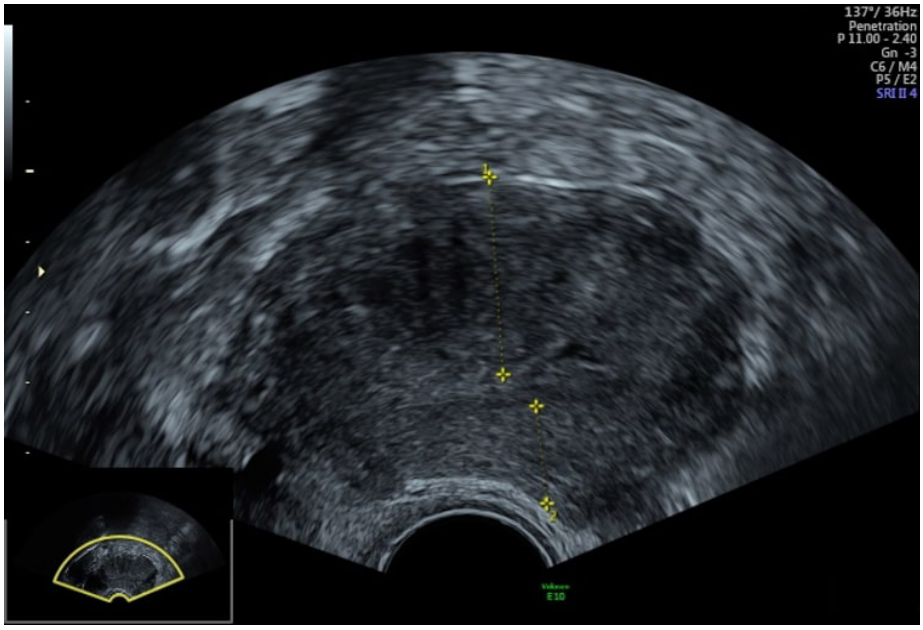

Figure 3a. Changes in focal adenomyosis I.

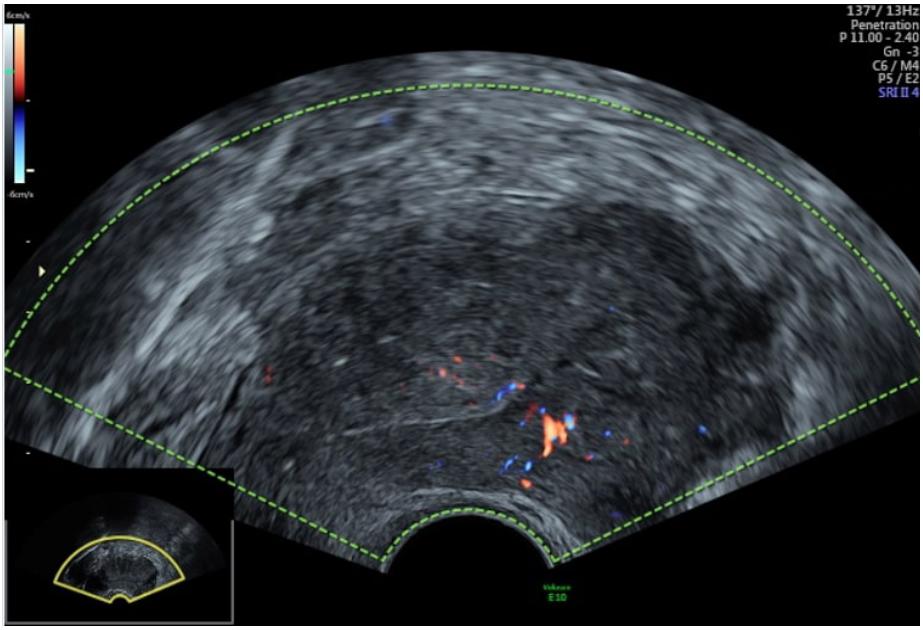

Figure 3b. Changes in focal adenomyosis II.

In this study, once adenomyosis was confirmed, we wanted to identify the risk factors that could lead to the development of this pathology.

Although it is considered that older age can have a direct impact on the appearance of the disease, among our patients we can see that most cases developed in women between 40 and 45 years old, more precisely 53.57% cases out of the 28 diagnosed (Figure 4).

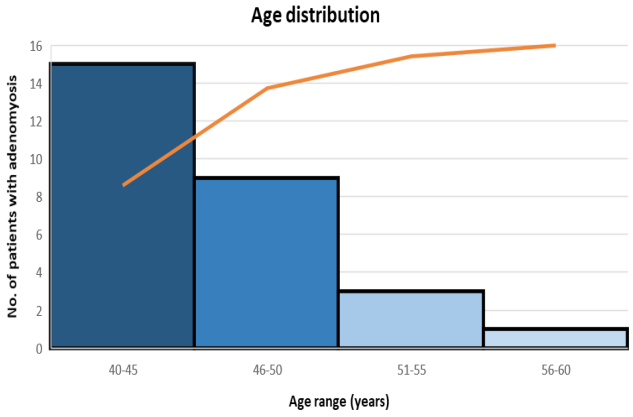

Figure 4. The influence of age on the occurrence of adenomyosis.

A first analysis was performed regarding the patients' environment of origin, it was compared whether the rural or urban environment can have a direct influence on the occurrence of adenomyosis. From our data, 15 cases of adenomyosis were observed from rural areas and 13 from urban areas. The area of origin could be considered a factor in the development of this pathology, but the results are insignificant (Figure 5).

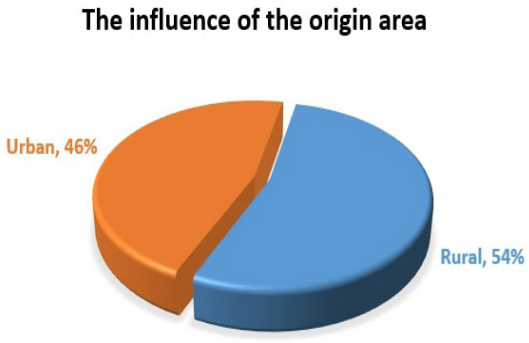

Figure 5. The influence of the origin area on the occurrence of adenomyosis.

A series of data were extracted from the patient's file, from symptomatology to personal history and comorbidities; based on which conclusions were drawn regarding the impact on the development of adenomyosis (Table II).

Table II. Clinical characteristics and personal antecedents.

Characteristics		Patients (n)	Patients (% of total)
Symptoms of adenomyosis patients	Dysmenorrhea + pelvic algae + dyspareunia	20	71.42
	Dysmenorrhea + pelvic algae	4	14.28
	Dyspareunia	3	10.71
	Asymptomatic	1	3.57
Pregnancies/childbirths	Multiparous	9	32.14
	Primiparous	17	60.71
	Nulliparous	2	7.14
The type of birth	Cesarean section	16	61.58
	Natural childbirth	7	26.92
Abortions	Abortion requested	8	28.57
	Biopsy curettage	5	17.85
	Pregnancy stopped in evolution, medical abortion (same patient)	1	3.57
	No history	14	50
Comorbidities	Genital + cardiovascular disorders	4	14.28
	Genital disorders	7	25
	Cardiovascular disorders	4	14.28
	Endocrine disorders	4	14.28
	Blood disorder	4	14.28
	Without associated pathologies	5	17.85
Treatment with progestogens	Levonorgestrel	10	35.71
	Progesteronum	4	14.28
	Dydrogesterone	3	10.71
	Lynestrenol	3	10.71
	Without treatment	8	28.57

In the present case, the patients complained of painful symptoms, such as dysmenorrhea, deep dyspareunia, and pelvic pain. One of the 28 women diagnosed with adenomyosis was asymptomatic, while the others presented in most cases all the other 3 symptoms (71.42%). Age, various uterine operations, and previous pregnancies can influence the appearance of adenomyosis. Thus, it was observed that the development of adenomyosis was associated with the number of births and was more frequent in the case of abortions. Multiple pregnancies can influence the development of this uterine pathology. In our study, out of the 28 patients diagnosed with adenomyosis, 32.14% of these are multiparous women, 60.71% are primiparous women and 7.14% are women without pregnancy up to that point. We notice that most women had only one pregnancy but were still diagnosed with adenomyosis. This aspect may be due to the fact that of the 17 primiparous women, 61.58% gave birth by cesarean section, and 3 of the patients had twin pregnancies, thus having a history of uterine operations. Therefore, it is not clear whether previous uterine surgical interventions act directly on the development of adenomyosis, but there is enough data to show that it can influence the appearance of this pathology. Moreover, among our patients, it became evident that the age between 40-45 years is the most prone to the occurrence of adenomyosis because half of them also went through an abortion, be it surgical, medicinal, or spontaneous.

As can be seen in the current study, most people diagnosed with adenomyosis also had other associated pathologies, the largest proportion being represented by urogenital disorders. Noting that most of the patients had at least three associated pathologies, we can say that coexisting pathologies can influence the development of adenomyosis.

***In vitro* analysis**

Since it is known that sex hormones can influence the development of breast cancer and observing that a large number of patients were receiving treatment with progestogens, we chose the most used molecule and tested it further *in vitro* on the triple-negative mammary cancer cell line.

Viability assessment

Initially, the MTT test was performed to evaluate the impact of LG on cell viability on the MDA-MB-231 cancer line after 24 and 48 hours of treatment. The obtained data show us that at the two concentrations tested, LG stimulates the proliferation of breast cancer cells depending on the concentration and exposure periods. Thus, after 24 hours, an increase in cell viability of up to 126% for the highest concentration was highlighted, and after 48 hours up to 120% for the concentration of 0.5 µM and 139% for 15 µM, as can be seen in Figure 6. We can state from the obtained results that LG can stimulate the proliferation of MDA-MB-231 breast cancer cells.

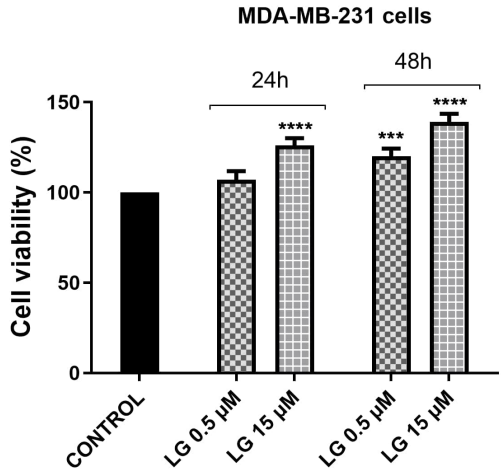

Figure 6. Viability percentage of MDA-MB-231 cells after treatment with LG (0.5 µM and 15 µM) at 24 h and 48 h post-exposure. The statistical differences between the control (untreated cells) and the treated cells (*** $p < 0.001$; **** $p < 0.0001$) were evaluated.

Cytotoxicity assay

To strengthen the results previously obtained with the help of the MTT method, we performed the LDH cytotoxicity test. According to the data, we observe that the tested concentrations of LG do not have a cytotoxic effect on MDA-MB-231 cells neither at 24 h nor at 48 h of stimulation. After a 48-hour stimulation, at the highest concentration a value of approximately -15% was obtained, which indicates that LG does not have a cytotoxic effect on the MDA-MB-231 tumor line (Figure 7).

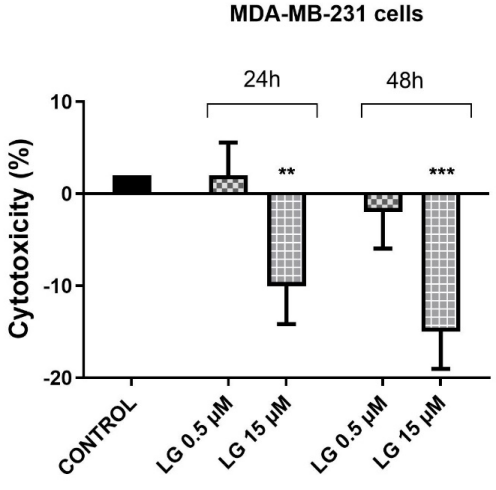

Figure 7. The potential cytotoxic of LG (0.5 µM and 15 µM) on the breast cancer MDA-MB-231 cell line at 24 h and 48 h post-exposure. The statistical differences between the control (untreated cells) and the treated cells (** $p < 0.01$, *** $p < 0.001$) were evaluated.

Discussion

Age (old age), is a risk factor for adenomyosis, demonstrated in various epidemiological studies, thus more than 70% of women undergoing hysterectomy after adenomyosis are in their fifth decade of life and multiparous [22-24]. However, adenomyosis can be found in women at the beginning of childbearing age [25]. Following a study conducted on women between 18 and 30 years old, it was found through ultrasound diagnosis that more than 30% of them show signs of adenomyosis, and in another study on women under 40 years old, it was observed through MRI, the presence of pathology in over 55% of cases [26,27].

The reports suggest that this pathology causes dysmenorrhea and pelvic pain in teenage girls [28], so we can conclude that the clinical age at the diagnosis of adenomyosis can be much earlier than previously estimated, as was also observed in the present study.

Our results can be correlated with the data from the literature, thus observing that the symptomatology known until now for adenomyosis is similar to that observed in the case of our patients.

Several studies claim that pelvic pain is common to adenomyosis, which occurs in approximately 50% of diagnosed women, and the intensity of the pain can be associated with the depth of myometrial invasion of ectopic endometrial areas [29,30]. Pelvic pains from adenomyosis can be due either to a weakened immune system that secretes neurogenic and algogenic substances [31] or due to increased contractility of the myometrium following the response to immune and hormonal changes [32]. Abundant menstruation and intermenstrual bleeding are common in patients with adenomyosis. It was found that the frequency of these bleedings increases the more the myometrium is affected by ectopic entities. Abnormal bleeding can be due to tissue damage, damage that occurs as a result of cyclic bleeding of the ectopic endometrium, which leads to inflammation and the activation and disruption of the vascular system at the local level [33].

Human studies indicate that adenomyosis could be associated with fertility impairment, but it is insufficiently described and the mechanisms involved are unclear. According to a meta-analysis, the prevalence of adenomyosis in infertile women undergoing fertilization varied from approximately 7% to 35% [34]. In animals, a closer link between adenomyosis and infertility has been observed. Thus, in a study conducted on 37 baboons with histologically confirmed adenomyosis, the prevalence of infertility was greatly increased in the presence of adenomyosis [35]. Infertility can be due to immunological changes in the ectopic endometrium, local estrogenic growth, alteration of cell proliferation, and cell adhesion to the endometrium, which lead to implantation abnormalities [16].

Studies in the literature report that a large percentage of women with adenomyosis are multiparous [22,23]. The possible explanation lies in the fact that pregnancy can facilitate the formation of the disease, by including the adenomyotic foci in the myometrium due to the invasive character of the trophoblast on the prolongation of the myometrial fibers [24]. Moreover, the adenomyotic tissue may have a greater number of estrogen receptors and the high hormonal titer may favor the development of the ectopic endometrium; knowing how many changes can occur in a pregnant woman [36].

There are studies in which women diagnosed with adenomyosis have not previously undergone any surgical intervention, there being no direct association between them [22,37]. However, in the study conducted by Whitted et al. an increased prevalence of adenomyosis was observed in patients who had previously undergone a cesarean section, data was also obtained in our study [38]. Moreover, other study exposed the fact that women who suffered from termination of pregnancy through different methods, the most common being curettage, presented higher rates of adenomyosis compared to women who did not go through such interventions [39].

The disease may present as one or a combination of thickening of the internal myometrium, areas of focal or diffuse disease, and involvement of the external myometrium. One idea is that trauma occurs at the endometrial myometrial interface encouraged by increased peristalsis. This process results in local trauma and a repair mechanism that leads to increased local estradiol levels that further accentuate hyperperistalsis and increased local damage that allows endometrial invasion into the myometrium [8,40].

Several ultrasonographic criteria have been used to diagnose adenomyosis, including the enlargement of the uterus, the asymmetry of the thickness of the uterine wall, the presence of heterogeneous myometrial areas, and the discovery of myometrial cysts [41]. In particular, the performed ultrasounds describe adenomyosis by highlighting myometrial cysts, by the presence of poorly defined myometrial heterogeneity, and by foci of abnormal myometrial echotexture.

Hysteroscopy is a major diagnostic and therapeutic tool for uterine conditions. The standard treatment for adenomyosis is hysterectomy, which was also used in our patients. However, this method cannot always be considered, especially when the woman wants to maintain her fertility or cannot undergo such surgery due to complications. Adenomyosis is a challenge in the field of gynecology and often hysterectomy is used to treat premenopausal and perimenopausal women. Hormonal suppressive treatments, such as oral contraceptives, high doses of progestogens, and hormone-releasing intrauterine devices, are known to temporarily stop the regression of the pathology and can be used in this sense [42], but may increase the risk of breast cancer [43], as highlighted in

our preliminary *in vitro* study. In our study, hysterectomy was resorted to with the approval of the patients, the great majority already having a child, being only two cases of nulliparous women.

The present *in vitro* study was conducted to investigate the impact of the synthetic sex hormone levonorgestrel on the behavior of MDA-MB-231 breast cancer cells, to identify a link in the development of breast cancer. The data reported that LG stimulates the proliferation of breast tumor cells depending on concentration and time; the highest values were observed when stimulated with the highest concentration of synthetic hormone after a 48-hour application.

Sex hormones play an important role in the etiopathogenesis of several types of cancer, including breast cancer [44]. Moreover, oral contraceptives with synthetic estrogens and progestogens are currently considered promoters of breast cancer progression [45]. In a study by Hasan et al., a concentration-dependent stimulatory effect of cell proliferation (1 pM-10 μM) of both progesterone and estrogen was evident on the MCF-7 breast cancer cell line after a 24-hour exposure h, but on the MDA-MB-231 cell line, the effect was not dependent on the dose [46]. In another study, a high percentage of cell proliferation was observed in two sex hormone receptor-positive breast cancer cell lines (HCC1500 and ZR75-1) following treatment with estradiol and ethinylestradiol, but also a negative influence on the expression of apoptotic markers and proliferative [47]. On the other hand, Zhou and co-workers obtained a concentration-dependent inhibition of the proliferation of MDA-MB-231 cells following the application of progesterone, but at lower concentrations (20-80 ng/mL) [48]. Moreover, the results obtained by Kadioğlu et al. showed that patients with adenomyosis present a high risk of high mammographic breast density [49]. This increase in breast density is closely related to the risk of breast cancer [50].

Conclusions

Clinically, adenomyosis is a heterogeneous disease, which differs from patient to patient. The specialized literature and our study reported the fact that clinical symptoms along with risk factors and more so personal antecedents can influence myometrial lesions, leading to diffuse or focal adenomyosis. Therefore, these characteristics may vary depending on the phenotype of the pathology.

Regarding the results of the *in vitro* evaluation of the synthetic sex hormone, levonorgestrel, we can state that it can stimulate the proliferation of MDA-MB-231 breast cancer cells at higher concentrations and with prolonged exposure. Moreover, the preliminary *in vitro* study is the first step in making a connection between the impact of adenomyosis in the development of breast cancer. However, this aspect requires a thorough evaluation in the future to be able to say with certainty if a factor that influences the appearance of adenomyosis has a direct impact on the development of breast cancer.

Finally, some limitations should be noted. The limitations of the present study refer to the small number of patients included and the heterogeneity of the risk factors highlighted in the group. Therefore, multicentric prospective studies are warranted.

References

1. Peric H, Fraser IS. The symptomatology of adenomyosis. Best Pract Res Clin Obstet Gynaecol. 2006;20:547-555.
2. Van den Bosch T, Van Schoubroeck D. Ultrasound diagnosis of endometriosis and adenomyosis: State of the art. Best Pract Res Clin Obstet Gynaecol. 2018;51:16-24.
3. Gunther R, Walker C. Adenomyosis. In: Gunther R, Walker C (Eds): StatPearls, StatPearls Publishing, 2023, Treasure Island, 1-16.
4. Upson K, Missmer SA. Epidemiology of Adenomyosis. Semin Reprod Med. 2020;38:89-107.
5. Bird CC, McElin TW, Manalo-Estrella P. The elusive adenomyosis of the uterus--revisited. Am J Obstet Gynecol. 1972;112:583-593.
6. Vannuccini S, Tosti C, Carmona F, Huang SJ, Chapron C, Guo SW, et al. Pathogenesis of adenomyosis: an update on molecular mechanisms. Reprod Biomed Online. 2017;35:592-601.
7. Harada T, Khine YM, Kaponis A, Nikellis T, Decavalas G, Taniguchi F. The Impact of Adenomyosis on Women's Fertility. Obstet Gynecol Surv. 2016;71:557-568.
8. Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. Hum Reprod Update. 2016;22:137-163.
9. Khan KN, Fujishita A, Koshiba A, Mori T, Kuroboshi H, Ogi H, et al. Biological differences between focal and diffuse adenomyosis and response to hormonal treatment. Reprod Biomed Online. 2019;38:634-646.
10. Manta L, Suciu N, Constantin A, Toader O, Popa F. Focal adenomyosis (intramural endometriotic cyst) in a very young patient - differential diagnosis with uterine fibromatosis. J Med Life. 2016;9:180-182.
11. Dueholm M, Lundorf E, Hansen ES, Sørensen JS, Ledertoug S, Olesen F. Magnetic resonance imaging and transvaginal ultrasonography for the diagnosis of adenomyosis. Fertil Steril. 2001;76:588–594.
12. Günther V, Allahqoli L, Gitas G, Maass N, Tesch K, Ackermann J, et al. Impact of Adenomyosis on Infertile Patients-Therapy Options and Reproductive Outcomes. Biomedicines. 2022;10:3245.
13. Baba A, Yamazoe S, Dogru M, Ogawa M, Takamatsu K, Miyauchi J. Clear cell adenocarcinoma arising from adenomyotic cyst: A case report and literature review. J Obstet Gynaecol Res. 2016;42:217-223.

14. Provendier A, Angeles MA, Meyrignac O, Illac C, Ducassou A, Martínez-Gómez C, et al. Clear cell adenocarcinoma arising from the abdominal wall after cesarean section in a patient with uterine adenomyosis. J Surg Case Rep. 2020;2020:rjaa070.
15. Moraru L, Mitranovici MI, Chiorean DM, Moraru R, Caravia L, Tiron AT, et al. Adenomyosis and Its Possible Malignancy: A Review of the Literature. Diagnostics (Basel). 2023;13:1883.
16. Bourdon M, Santulli P, Jeljeli M, Vannuccini S, Marcellin L, Doridot L, et al. Immunological changes associated with adenomyosis: a systematic review. Hum Reprod Update. 2021;27:108-129.
17. Hilborn E, Stål O, Jansson A. Estrogen and androgen-converting enzymes 17β-hydroxysteroid dehydrogenase and their involvement in cancer: with a special focus on 17βhydroxysteroid dehydrogenase type 1, 2, and breast cancer. Oncotarget. 2017;8:30552-30562.
18. Harbeck N, Gnant M. Breast cancer. Lancet. 2017;389:1134-1150.
19. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer (Dove Med Press). 2019;11:151-164.
20. Şoica C, Voicu M, Ghiulai R, Dehelean C, Racoviceanu R, Trandafirescu C, et al. Natural Compounds in Sex Hormone-Dependent Cancers: The Role of Triterpenes as Therapeutic Agents. Front Endocrinol (Lausanne). 2021;11:612396.
21. Hilton HN, Clarke CL, Graham JD. Estrogen and progesterone signalling in the normal breast and its implications for cancer development. Mol Cell Endocrinol. 2018;466:2-14.
22. Taran FA, Weaver AL, Coddington CC, Stewart EA. Understanding adenomyosis: a case control study. Fertil Steril. 2010;94:1223-1228.
23. Taran FA, Wallwiener M, Kabashi D, Rothmund R, Rall K, Kraemer B, et al. Clinical characteristics indicating adenomyosis at the time of hysterectomy: a retrospective study in 291 patients. Arch Gynecol Obstet. 2012;285:1571-1576.
24. Garcia L, Isaacson K. Adenomyosis: review of the literature. J Minim Invasive Gynecol. 2011;18:428-437.
25. Yu O, Schulze-Rath R, Grafton J, Hansen K, Scholes D, Reed SD. Adenomyosis incidence, prevalence and treatment: United States population-based study 2006-2015. Am J Obstet Gynecol. 2020;223:94.e1-94.e10.
26. Pinzauti S, Lazzeri L, Tosti C, Centini G, Orlandini C, Luisi S, et al. Transvaginal sonographic features of diffuse adenomyosis in 18-30-year-old nulligravid women without endometriosis: association with symptoms. Ultrasound Obstet Gynecol. 2015;46:730-736.
27. Chapron C, Tosti C, Marcellin L, Bourdon M, Lafay-Pillet MC, Millischer AE, et al. Relationship between the magnetic resonance imaging appearance of adenomyosis and endometriosis phenotypes. Hum Reprod. 2017;32:1393-1401.
28. Ryan GL, Stolpen A, Van Voorhis BJ. An unusual cause of adolescent dysmenorrhea. Obstet Gynecol. 2006;108(4):1017-1022.
29. Bourdon M, Oliveira J, Marcellin L, Santulli P, Bordonne C, Maitrot Mantelet L, et al. Adenomyosis of the inner and outer myometrium are associated with different clinical profiles. Hum Reprod. 2021;36:349-357.
30. Exacoustos C, Morosetti G, Conway F, Camilli S, Martire FG, Lazzeri L, et al. New Sonographic Classification of Adenomyosis: Do Type and Degree of Adenomyosis Correlate to Severity of Symptoms? J Minim Invasive Gynecol. 2020;27:1308-1315.
31. Carrarelli P, Yen CF, Funghi L, Arcuri F, Tosti C, Bifulco G, et al. Expression of Inflammatory and Neurogenic Mediators in Adenomyosis. Reprod Sci. 2017;24:369-375.
32. Kunz G, Leyendecker G. Uterine peristaltic activity during the menstrual cycle: characterization, regulation, function and dysfunction. Reprod Biomed Online. 2002;4 Suppl 3:5-9.
33. Harmsen MJ, Wong CFC, Mijatovic V, Griffioen AW, Groenman F, Hehenkamp WJK, et al. Role of angiogenesis in adenomyosis-associated abnormal uterine bleeding and subfertility: a systematic review. Hum Reprod Update. 2019;25:647-671.
34. Vercellini P, Consonni D, Dridi D, Bracco B, Frattaruolo MP, Somigliana E. Uterine adenomyosis and in vitro fertilization outcome: a systematic review and meta-analysis. Hum Reprod. 2014;29:964-977.
35. Barrier BF, Malinowski MJ, Dick EJ Jr, Hubbard GB, Bates GW. Adenomyosis in the baboon is associated with primary infertility. Fertil Steril. 2004;82 Suppl 3:1091-1094.
36. Fiat F, Merghes PE, Scurtu AD, Almajan Guta B, Dehelean CA, Varan N, et al. The Main Changes in Pregnancy-Therapeutic Approach to Musculoskeletal Pain. Medicina (Kaunas). 2022;58:1115.
37. Bergholt T, Eriksen L, Berendt N, Jacobsen M, Hertz JB. Prevalence and risk factors of adenomyosis at hysterectomy. Hum Reprod. 2001;16:2418-2421.
38. Whitted R, Verma U, Voigl B, Mendez L. Does cesarean delivery increase the prevalence of adenomyosis? A retrospective review. Obstet Gynecol. 2000;95:S83.
39. Parazzini F, Mais V, Cipriani S, Busacca M, Venturini P, GISE. Determinants of adenomyosis in women who underwent hysterectomy for benign gynecological conditions: results from a prospective multicentric study in Italy. Eur J Obstet Gynecol Reprod Biol. 2009;143:103-106.
40. Chen YJ, Li HY, Huang CH, Twu NF, Yen MS, Wang PH, et al. Oestrogen-induced epithelial-mesenchymal transition of endometrial epithelial cells contributes to the development of adenomyosis. J Pathol. 2010;222:261–270.
41. Graziano A, Lo Monte G, Piva I, Caserta D, Karner M, Engl B, et al. Diagnostic findings in adenomyosis: a pictorial review on the major concerns. Eur Rev Med Pharmacol Sci. 2015;19:1146–1154.
42. Taran FA, Stewart EA, Brucker S. Adenomyosis: Epidemiology, Risk Factors, Clinical Phenotype and Surgical and Interventional Alternatives to Hysterectomy. Geburtshilfe Frauenheilkd. 2013;73:924-931.
43. Trabert B, Sherman ME, Kannan N, Stanczyk FZ. Progesterone and Breast Cancer. Endocr Rev. 2020;41:320–

344.

44. Folkerd E, Dowsett M. Sex hormones and breast cancer risk and prognosis. Breast. 2013;22 Suppl 2:S38-S43.
45. Bardaweel SK, Akour AA, Al-Muhaissen S, AlSalamat HA, Ammar K. Oral contraceptive and breast cancer: do benefits outweigh the risks? A case - control study from Jordan. BMC Womens Health. 2019;19:72.
46. Hasan M, Browne E, Guarinoni L, Darveau T, Hilton K, Witt-Enderby PA. Novel Melatonin, Estrogen, and Progesterone Hormone Therapy Demonstrates Anti-Cancer Actions in MCF-7 and MDA-MB-231 Breast Cancer Cells. Breast Cancer (Auckl). 2020;14:1178223420924634.
47. Merki-Feld GS, Seeger H, Mueck AO. Proliferative effects of estradiol- or ethinylestradiol-progestogen combinations on human breast cancer cells in an intermitted and a long-term regimen. Horm Metab Res. 2012;44:415-421.
48. Zhou L, Zhou W, Zhang H, Hu Y, Yu L, Zhang Y, et al. Progesterone suppresses triple-negative breast cancer growth and metastasis to the brain via membrane progesterone receptor α. Int J Mol Med. 2017;40:755-761.
49. Kadioğlu N, Yorganci A, Gümgümcü H, Kokanali MK, Özyer Ş, Üstün Y. Evaluation Of Mammographic Features In Women With Adenomyosis. J Exp Clin Med. 2022;39:989-998.
50. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2006;15:1159-1169.